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(54) Title: DNA ENCODING A $GABA_BR2$ POLYPEPTIDE AND USES THEREOF

(57) Abstract

This invention provides isolated nucleic acids encoding a mammalian $GABA_BR2$ polypeptide, an isolated $GABA_BR2$ protein, vectors comprising isolated nucleic acid encoding mammalian $GABA_BR2$ polypeptides, cells expressing mammalian $GABA_BR1/R2$ receptors, antibodies directed to an epitope on mammalian $GABA_BR2$ polypeptides or mammalian $GABA_BR1/R2$ receptors, nucleic acid probes useful for detecting nucleic acids encoding mammalian $GABA_BR2$ polypeptides, antisense oligonucleotides complementary to unique sequences of nucleic acids encoding mammalian $GABA_BR2$ polypeptides, nonhuman transgenic animals which express DNA encoding normal or mutant mammalian $GABA_BR1/R2$ receptors, as well as methods of screening compounds acting as agonists or antagonists of mammalian $GABA_BR1/R2$ receptors.

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DNA ENCODING A $GABA_B$R2 POLYPEPTIDE AND USES THEREOF

BACKGROUND OF THE INVENTION

This application is a continuation-in-part of U.S. Serial No. 09/141,760, filed August 27, 1998, which is a continuation-in-part of U.S. Serial No. 08/953,277, filed October 17, 1997, the contents of which are hereby incorporated by reference into the subject application.

Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found at the end of this application, preceding the sequence listing and the claims.

Gamma amino butyric acid (GABA) is the major inhibitory neurotransmitter in the nervous system. Three families of receptors for this neurotransmitter, $GABA_A$, $GABA_B$, and $GABA_C$, have been defined pharmacologically and genetically. $GABA_B$ receptors were initially discriminated by their sensitivity to the drug baclofen (Bowery, 1993). This and their dependency on G-proteins for effector coupling distinguishes them from the ion channel-forming $GABA_A$ and $GABA_C$ receptors. Principle molecular targets of $GABA_B$ receptor activation are Ca^{++} and K^+ channels whose gating is directly modulated by the liberation of G-protein that follows the binding of the neurotransmitter to its receptor (Misgeld et al. 1995; Krapivinsky et al., 1995a). In this sense, $GABA_B$ receptors operate mechanistically as other G-protein coupled receptors (GPCRs), such as dopamine D2, serotonin 5HT1a, neuropeptide Y and opiate receptors, that are also negatively coupled to adenylyl cyclase activity (North, 1989). Stimulation of $GABA_B$ receptors inhibits release

of neurotransmitters such as glutamate, GABA, somatostatin, and acetylcholine by modulation of Ca^{++} and K^{+} channels at presynaptic nerve terminals. Inhibition of neurotransmitter release is one of the most prominent physiological actions of the $GABA_B$ receptor and has provided a basis for the discrimination of receptor subtypes (Bowery et al. 1990). $GABA_B$ receptors also mediate a powerful postsynaptic hyperpolarization of neuronal cell bodies via the opening of G-protein-gated inwardly rectifying K^{+} channels (GIRK) (Kofuji et al. 1996).

$GABA_B$ receptors are widely distributed throughout the central nervous system. Receptor autoradiography and binding studies show that receptors are found in relatively high abundance in nearly all areas of the brain including cerebral cortex, hippocampus, cerebellum, basal ganglia, thalamus, and spinal cord (Bowery et al. 1987). In the periphery, GABA and $GABA_B$ receptors are found in pancreatic islets, autonomic ganglia, guinea-pig ileum, lung, oviduct, and urinary bladder (Giotti et al. 1983; Erdo et al. 1984; Santicioli et al. 1986; Sawynok, 1986; Hills et al. 1989; Chapman et al. 1993).

Baclofen, the agonist that originally defined the $GABA_B$ receptor subtype, has been used as an anti-spastic agent for the past 25 years. There is evidence in human that baclofen has a spinal site of action that most likely involves the depression of mono-and polysynaptic reflexes. In laboratory animals, baclofen has antinociceptive properties that are attributed to the inhibition of release of excitatory neurotransmitters glutamate and substance P from primary sensory afferent terminals (Dirig and Yaksh, 1978; Sawynok, 1987; Malcangio et al., 1991). The presence of $GABA_B$ receptors in intestine, lung and urinary bladder indicates a possible therapeutic role for diseases associated with these peripheral tissues. In spinal patients, baclofen is currently used for

treatment of bladder-urethral dissynergia (Leyson et al., 1980). Selective $GABA_B$ receptor agonists may also prove useful for the treatment of incontinence by reducing the feeling of bladder fullness (Taylor and Bates, 1979). Evidence from studies of the upper respiratory systems of cats and guinea-pigs suggests that $GABA_B$ agonists also may be useful as antitussive agents and for the treatment of asthma (Luzzi et al., 1987; Bolser et al., 1993). In addition, $GABA_B$ receptors have been implicated in absence seizure activity in the neocortex and with presynaptic depression of excitatory transmission in the spinal cord.

Studies of $GABA_B$ receptor pharmacology and physiology have been greatly facilitated by the relatively recent arrival of potent and selective $GABA_B$ receptor antagonists that are able to penetrate the blood-brain barrier. The most fruitful avenue for providing glimpses of $GABA_B$ receptor subtypes has come from studies of neurotransmitter release. GABA, acting through $GABA_B$ receptors, can inhibit the release of GABA, glutamate, and somatostatin in rat cerebrocortical synaptosomes depolarized with KCl. Three receptor subtypes have been hypothesized based on the potency of the agonists baclofen and 3-aminopropylphosphinic acid (3-APPA), and on the antagonists phaclofen and CGP35348 (Bonanno, Raiteri, 1992). For example, somatostatin release is inhibited by baclofen and this effect is antagonized by phaclofen and CGP35348. Glutamate release is similarly affected except that the potency of phaclofen to block inhibition is considerably lower than that for release of somatostatin. A third receptor subtype, the cortical GABA autoreceptor, has been defined based on an insensitivity to CGP35348, although this potency difference is not seen in a cortical slice preparation (Waldmeier et al. 1994). In the spinal cord, the GABA autoreceptor is insensitive to baclofen, but sensitive to 3APPA and block by CGP35348. Interestingly, in this tissue baclofen is active at the $GABA_B$ receptor

modulating glutamate release. Differences in the sensitivities of presynaptic receptors controlling release of GABA and glutamate in the spinal cord may importantly contribute to the therapeutic action of baclofen as an antispastic agent (Bonanno, Raiteri, 1993).

Recently a polypeptide was isolated, $GABA_BR1a$, that binds radiolabelled $GABA_B$ receptor antagonists in transfected cells (Kaupmann et al. 1997a). The predicted amino acid sequence displays homology with the metabotropic glutamate receptor gene family which includes eight members and a Ca^{++}-sensing receptor. Included in this homology is a large N-terminal domain that contains two lobes with structural similarity to the amino acid binding sites of bacterial proteins. A second polypeptide, $GABA_BR1b$, presumably a splice variant, differs from $GABA_BR1a$ in that the N-terminal 147 amino acids are replaced by 18 different residues in the predicted mature protein after signal peptide cleavage. Transcripts for both $GABA_BR1s$ are abundant and widely distributed in the rat brain. There appear to be differences in the localization of the splice variants in discrete regions of the brain, suggesting that their expression is differentially regulated (Bischoff et al. 1997).

The pharmacological profile of the cloned $GABA_BR1$ polypeptide is similar in some respects to that of native receptors isolated from rat cerebral cortex, but there are important differences. For the high affinity antagonists studied, IC_{50}s are nearly identical to those at native receptors. In contrast, IC_{50}s for agonists and some low affinity antagonists display large rightward shifts relative to their displacement curves in native tissue. Additionally, both splice variants of the polypeptide couple poorly to intracellular effectors such as inhibition of adenylyl cyclase and, against expectations, fail completely to stimulate GIRK currents in

oocytes (Kaupmann et al. 1997b). The poor binding affinity of agonists and weak or non-existent activation of effectors may not be adequately explained by inappropriate G-protein coupling in the heterologous expression system used.

The isolation by homology cloning of a novel polypeptide, $GABA_BR2$, from a human hippocampus cDNA library, as well the isolation of the rat homolog of the human polypeptide, is now reported. Also reported herein are functional assays involving the co-expression of the $GABA_BR2$ gene with a $GABA_BR1$ gene. These functional assays were not previously observed with the $GABA_BR1$ gene product alone. The pharmacological and signal transduction properties of the two gene products when expressed together match those of native $GABA_B$ receptors in the brain. These functional assays permits high throughput screening for novel compounds having agonist or antagonist activity at the native $GABA_B$ receptor.

SUMMARY OF THE INVENTION

This invention is directed to an isolated nucleic acid encoding a $GABA_BR2$ polypeptide.

This invention is further directed to a purified $GABA_BR2$ protein.

This invention is further directed to a vector comprising the above-identified nucleic acid.

This invention is further directed to a above-identified vector, wherein the vector is a plasmid.

This invention is directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within one of the two strands of the nucleic acid encoding the $GABA_BR2$ polypeptide contained in plasmid BO-55, and detecting hybridization of the probe to the nucleic acid.

This invention is further directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within (a) the nucleic acid sequence shown in Figures 1A-1E (Seq. ID No. 1) or (b) the reverse complement to the nucleic acid sequence shown in Figures 1A-1E (Seq. ID No. 1), and detecting hybridization of the probe to the nucleic acid.

This invention is further directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises

contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within one of the two strands of the nucleic acid encoding the $GABA_BR2$ polypeptide contained in plasmid TL-267, and detecting hybridization of the probe to the nucleic acid.

This invention is further directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within (a) the nucleic acid sequence shown in Figures 3A-3D (Seq. ID No. 3) or (b) the reverse complement to the nucleic acid sequence shown in Figures 3A-3D (Seq. ID No. 3), and detecting hybridization of the probe to the nucleic acid.

This invention is further directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising a nucleic acid of at least 15 nucleotides which is complementary to the antisense sequence of a unique segment of the sequence of the nucleic acid encoding the $GABA_BR2$ polypeptide, and detecting hybridization of the probe to the nucleic acid.

This invention is directed to an isolated antibody capable of binding to a $GABA_BR2$ polypeptide encoded by the above-identified nucleic acid.

This invention is further directed to an antibody capable of competitively inhibiting the binding of the above-identified antibody to a $GABA_BR2$ polypeptide.

This invention is further directed to a pharmaceutical composition which comprises an amount of the above-identified

antibody effective to block binding of a ligand to the $GABA_BR2$ polypeptide and a pharmaceutically acceptable carrier.

This invention is directed to a transgenic, nonhuman mammal expressing DNA encoding a $GABA_BR2$ polypeptide.

This invention is further directed to a transgenic, nonhuman mammal comprising a homologous recombination knockout of the native $GABA_BR2$ polypeptide.

This invention is further directed to a transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding an above-identified $GABA_BR2$ polypeptide so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding such $GABA_BR2$ polypeptide and which hybridizes to such mRNA encoding such $GABA_BR2$ polypeptide, thereby reducing its translation.

This invention is directed to a method of detecting the presence of a $GABA_BR2$ polypeptide on the surface of a cell which comprises contacting the cell with the above-identified antibody under conditions permitting binding of the antibody to the polypeptide, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a $GABA_BR2$ polypeptide on the surface of the cell.

This invention is further directed to a method of preparing the purified $GABA_BR2$ polypeptide which comprises:

a. inducing cells to express a $GABA_BR2$ polypeptide;

b. recovering the polypeptide so expressed from the induced cells; and

c. purifying the polypeptide so recovered.

This invention is further directed to a method of preparing

the purified $GABA_BR2$ polypeptide which comprises:

a. inserting a nucleic acid encoding the $GABA_BR2$ polypeptide into a suitable vector;

b. introducing the resulting vector in a suitable host cell;

c. placing the resulting cell in suitable condition permitting the production of the $GABA_BR2$ polypeptide;

d. recovering the polypeptide produced by the resulting cell; and

e. isolating or purifying the polypeptide so recovered.

This invention is directed to a $GABA_BR1/R2$ receptor comprising two polypeptides, one of which is a $GABA_BR2$ polypeptide and another of which is a $GABA_BR1$ polypeptide.

This invention is directed to a method of forming a $GABA_BR1/R2$ receptor which comprises inducing cells to express both a $GABA_BR1$ polypeptide and a $GABA_BR2$ polypeptide.

This invention is directed to an antibody capable of binding to a $GABA_BR1/R2$ receptor, wherein the $GABA_BR2$ polypeptide is encoded by the above-identified nucleic acid.

This invention is further directed to an antibody capable of competitively inhibiting the binding of the above-identified antibody to a $GABA_BR1/R2$ receptor.

This invention is directed to a pharmaceutical composition which comprises an amount of the above-identified antibody effective to block binding of a ligand to the $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

This invention is directed to a transgenic, nonhuman mammal expressing a $GABA_BR1/R2$ receptor, which is not naturally expressed by the mammal.

This invention is further directed to a transgenic, nonhuman mammal comprising a homologous recombination knockout of the native $GABA_BR1/R2$ receptor.

This invention is directed to a method of detecting the presence of a $GABA_BR1/R2$ receptor on the surface of a cell which comprises contacting the cell with the above-identified antibody under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a $GABA_BR1/R2$ receptor on the surface of the cell.

This invention is directed to a method of determining the physiological effects of varying levels of activity of $GABA_BR1/R2$ receptors which comprises producing an above-identified transgenic nonhuman mammal whose levels of $GABA_BR1/R2$ receptor activity vary due to the presence of an inducible promoter which regulates $GABA_BR1/R2$ receptor expression.

This invention is directed to a cell which expresses on its surface a mammalian $GABA_BR1/R2$ receptor that is not naturally expressed on the surface of such cell.

This invention is directed to a process for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor.

This invention is directed to a process for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises contacting a membrane fraction from a cell extract of cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor.

This invention is directed to a process involving competitive binding for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises separately contacting cells expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor, a decrease in the binding of the second chemical compound to the $GABA_BR1/R2$ receptor in the presence of the chemical compound indicating that the chemical compound binds to the $GABA_BR1/R2$ receptor.

This invention is directed to a process involving competitive binding for identifying a chemical compound which specifically binds to a human $GABA_BR1/R2$ receptor which comprises separately contacting a membrane fraction from a cell extract of cells expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor, a decrease in the binding of the second chemical compound to the $GABA_BR1/R2$

receptor in the presence of the chemical compound indicating that the chemical compound binds to the $GABA_BR1/R2$ receptor.

This invention is directed to a method of screening a plurality of chemical compounds not known to bind to a $GABA_BR1/R2$ receptor to identify a compound which specifically binds to the $GABA_BR1/R2$ receptor, which comprises

(a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with a compound known to bind specifically to the $GABA_BR1/R2$ receptor;

(b) contacting the same cells as in step (a) with the plurality of compounds not known to bind specifically to the $GABA_BR1/R2$ receptor, under conditions permitting binding of compounds known to bind the $GABA_BR1/R2$ receptor;

(c) determining whether the binding of the compound known to bind specifically to the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of the compounds, relative to the binding of the compound in the absence of the plurality of compounds, and if the binding is reduced;

(d) separately determining the extent of binding to the $GABA_BR1/R2$ receptor of each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which specifically binds to the $GABA_BR1/R2$ receptor.

This invention is directed to a method of screening a plurality of chemical compounds not known to bind to a $GABA_BR1/R2$ receptor to identify a compound which specifically

binds to the $GABA_BR1/R2$ receptor, which comprises

(a) contacting a membrane fraction extract from cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with a compound known to bind specifically to the $GABA_BR1/R2$ receptor;

(b) contacting the same membrane fraction as in step (a) with the plurality of compounds not known to bind specifically to the $GABA_BR1/R2$ receptor, under conditions permitting binding of compounds known to bind the $GABA_BR1/R2$ receptor;

(c) determining whether the binding of the compound known to bind specifically to the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds, and if the binding is reduced;

(d) separately determining the extent of binding to the $GABA_BR1/R2$ receptor of each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which specifically binds to the $GABA_BR1/R2$ receptor.

This invention is directed to a process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor agonist which comprises contacting cells with the compound under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting an increase in $GABA_BR1/R2$ receptor activity, so as to thereby determine whether the compound is a $GABA_BR1/R2$ receptor agonist.

This invention is directed to a process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor antagonist which comprises contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the compound in the presence of a known $GABA_BR1/R2$ receptor agonist, under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting a decrease in $GABA_BR1/R2$ receptor activity, so as to thereby determine whether the compound is a $GABA_BR1/R2$ receptor antagonist.

This invention is directed to a process for determining whether a chemical compound activates a $GABA_BR1/R2$ receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the chemical compound under conditions suitable for activation of the $GABA_BR1/R2$ receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the $GABA_BR1/R2$ receptor.

This invention is directed to a process for determining whether a chemical compound inhibits activation of a $GABA_BR1/R2$ receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with both the chemical compound and a second chemical compound known to activate the $GABA_BR1/R2$ receptor, and with only the second chemical compound, under conditions suitable for activation of the $GABA_BR1/R2$ receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and

the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the $GABA_BR1/R2$ receptor.

This invention is directed to a method of screening a plurality of chemical compounds not known to activate a $GABA_BR1/R2$ receptor to identify a compound which activates the $GABA_BR1/R2$ receptor which comprises:

(a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the plurality of compounds not known to activate the $GABA_BR1/R2$ receptor, under conditions permitting activation of the $GABA_BR1/R2$ receptor;

(b) determining whether the activity of the $GABA_BR1/R2$ receptor is increased in the presence of the compounds, and if it is increased;

(c) separately determining whether the activation of the $GABA_BR1/R2$ receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which activates the $GABA_BR1/R2$ receptor.

This invention is directed to a method of screening a plurality of chemical compounds not known to inhibit the activation of a $GABA_BR1/R2$ receptor to identify a compound which inhibits the activation of the $GABA_BR1/R2$ receptor, which comprises:

(a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the plurality of compounds in the presence of a known $GABA_BR1/R2$ receptor agonist, under conditions permitting activation of the $GABA_BR1/R2$ receptor;

(b) determining whether the activation of the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of compounds, relative to the activation of the $GABA_BR1/R2$ receptor in the absence of the plurality of compounds, and if it is reduced;

(c) separately determining the inhibition of activation of the $GABA_BR1/R2$ receptor for each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such a plurality of compounds which inhibits the activation of the $GABA_BR1/R2$ receptor.

This invention is directed to a process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor agonist, which comprises preparing a membrane fraction from cells which comprise nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, separately contacting the membrane fraction with both the chemical compound and GTPγS, and with only GTPγS, under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting GTPγS binding to the membrane fraction, an increase in GTPγS binding in the presence of the compound indicating that the chemical compound activates the $GABA_BR1/R2$ receptor.

This invention is directed to aprocess for determining whether a chemical compound is a $GABA_BR1/R2$ receptor antagonist, which comprises preparing a membrane fraction from cells which

comprise nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, separately contacting the membrane fraction with the chemical compound, GTPγS and a second chemical compound known to activate the $GABA_BR1/R2$ receptor, with GTPγS and only the second compound, and with GTPγS alone, under conditions permitting the activation of the $GABA_BR1/R2$ receptor, detecting GTPγS binding to each membrane fraction, and comparing the increase in GTPγS binding in the presence of the compound and the second compound relative to the binding of GTPγS alone, to the increase in GTPγS binding in the presence of the second chemical compound known to activate the $GABA_BR1/R2$ receptor relative to the binding of GTPγS alone, a smaller increase in GTPγS binding in the presence of the compound and the second compound indicating that the compound is a $GABA_BR1/R2$ receptor antagonist.

This invention is directed to a method of treating spasticity in a subject which comprises administering to the subject an amount of a compound which is an agonist of a $GABA_BR1/R2$ receptor effective to treat spasticity in the subject.

This invention is directed to a method of treating asthma in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to treat asthma in the subject.

This invention is directed to a method of treating incontinence in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to treat incontinence in the subject.

This invention is directed to a method of decreasing nociception in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to decrease nociception in the subject.

This invention is directed to a use of a $GABA_BR2$ agonist as an antitussive agent which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective as an antitussive agent in the subject.

This invention is directed to a method of treating drug addiction in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to treat drug addiction in the subject.

This invention is directed to a method of treating Alzheimer's disease in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor antagonist effective to treat Alzheimer's disease in the subject.

This invention is directed to a peptide selected from the group consisting of:

a) P L Y S I L S A L T I L G M I M A S A F L F F N I K N;
b) L I I L G G M L S Y A S I F L F G L D G S F V S E K T;
c) C T V R T W I L T V G Y T T A F G A M F A K T W R;
d) Q K L L V I V G G M L L I D L C I L I C W Q;
e) M T I W L G I V Y A Y K G L L M L F G C F L A W;
f) A L N D S K Y I G M S V Y N V G I M C I I G A A V; and
g) C I V A L V I I F C S T I T L C L V F V P K L I T L R T N .

This invention is directed to a compound that prevents the formation of a $GABA_BR1/R2$ receptor complex.

Finally, this invention provides a process for making a composition of matter which specifically binds to a $GABA_BR1/R2$ receptor which comprises identifying a chemical compound using any of the processes described herein for identifying a compound which binds to and/or activates or inhibits

activation of a $GABA_BR1/R2$ receptor and then synthesizing the chemical compund or a novel structural and functional analog or homolog thereof. This invention furhter provides a process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by any of the processes described herein for identifying a compound which binds to and/or activates or inhibits activation of a $GABA_BR1/R2$ receptor or a novel structural and functional analog or homolog thereof.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1E Nucleotide coding sequence of the human $GABA_BR2$ polypeptide (Seq. ID No. 1), with partial 5' and 3' untranslated sequences. Two possible start (ATG) codons are underlined as well as the stop codon (TAA).

Figures 2A-2D Deduced amino acid sequence of the human $GABA_BR2$ polypeptide (Seq. ID No. 2) encoded by the nucleotide sequence shown in Figures 1A-1E.

Figures 3A-3D Nucleotide coding sequence of the rat $GABA_BR2$ polypeptide (Seq. ID No. 3). Start (ATG) and stop (TAG) codons are underlined.

Figures 4A-4D Deduced amino acid sequence of the rat $GABA_BR2$ polypeptide (Seq. ID No. 4) encoded by the nucleotide sequence shown in Figures 3A-3D.

Figures 5A-5D Amino acid sequence of the human $GABA_BR2$ polypeptide (Seq. ID No. 2) with brackets above the sequence showing the boundaries of seven (7) putative transmembrane domains, numbered consecutively from I to VII.

Figures 6A-6B. Measurement of EC_{50} for GABA in a cumulative concentration response assay in oocytes expressing $GABA_BR1b/GABA_BR2$ + GIRKs. Figure 6A: Electrophysiological trace from a voltage clamped oocyte showing increasing inward currents evoked successively by concentrations of GABA ranging from 0.03 to 30 µM. Numbers over bars indicate concentration of GABA in µM. hK is 49 mM external K^+. Figure 6B: Averaged responses from 3-6 oocytes plotted *vs.* concentration of GABA results in an EC_{50} value of 1.76 µM. For each oocyte, currents were normalized to the maximum response at 30 µM.

Figure 7. Concentration response relationship for baclofen in oocytes expressing $GABA_BR1b/GABA_BR2$ + GIRKs. Methods are as described for Figure 6.

Figure 8. Current voltage relationship for the current activated by GABA in oocytes expressing $GABA_BR1b/GABA_BR2$ + GIRKs. Voltage ramps (50 mV/s) from -140 to +40 mV were applied in the presence of GABA (in hK) and again in the presence of GABA + 100 μM Ba^{++} to block inward rectifier current. The resulting traces were subtracted (GABA alone - GABA + Ba^{++}) to yield the Ba^{++}-sensitive portion of the GABA-stimulated current. As expected for GIRK current, the current displays steep inward rectification and reverses near the predicted equilibrium potential for K+ (-23 mV in hK).

Figures 9A-9B. Electrophysiological responses under voltage clamp conditions to GABA in an HEK-293 cell transiently transfected with $GABA_BR1b/GABA_BR2$ + GIRKs. A) The continuous trace (in presence of 25 mM K^+) shows a small constitutive inward rectifier current that is blocked by Ba^{++} (100 μM), and a much larger inward current induced by application of GABA that is also blocked by Ba^{++}. A second GABA-evoked current is abolished by the selective antagonist CGP55845. After a 1 minute wash period GABA-responsivity returns. B) Concentration response relation for GABA in 5 HEK-293 cells expressing $GABA_BR1b/GABA_BR2$ + GIRKs. (See Figure 6B for details.)

Figure 10. Alignment of amino acid sequences predicted for rat $GABA_BR2$ and rat $GABA_BR1$. Shaded regions highlight sequence identities. Horizontal bars indicate TM regions.

Figures 11A-11D. Photomicrographs showing the regional distribution of the $GABA_BR1$ (A,C) and $GABA_BR2$ (B,D) mRNAs in representative coronal rat brain sections. Hypothalamus and caudate-putamen are identified with arrow heads and arrows, respectively (A,B). Arrows identify Purkinje cell layer in cerebellum (C,D).

Figures 12A-12B. High magnification micrographs of Purkinje cell layer from alternate serial sections showing co-localization of $GABA_BR2$ transcripts using digoxigenin-labeled probes (A) and $GABA_BR1$ transcripts using [^{35}S]dATP-labeled probes (B) in the same cells (asterisks). Scale bar = 30 µM.

Figures 13A-13B. Figure 13A: Response to GABA (100 µM) from oocyte expressing $GABA_BR1$, $GABA_BR2$, and GIRKs (lower trace). Similar oocyte pretreated 6 h earlier with pertussis toxin (2 ng injected; upper trace). Figure 13B: Summary of mean response amplitudes from oocytes expressing various combinations of $GABA_BR1$ and $GABA_BR2$ plus GIRKs. Responses are to 100 µM GABA (solid bars) or 100 µM baclofen (open bar). Number of observations are in parenthesis.

Figures 14A-14B. Figure 14A: Response to GABA or baclofen (100 µM in 25 mM K^+) in HEK293 cells expressing GIRKs along with $GABA_BR1b$, $GABA_BR2$, or both. Figure 14B: Summary of mean response amplitudes from HEK293 cells co-transfected with various combinations and ratios of cDNA. To prepare different ratios of $GABA_BR1b$:$GABA_BR2$ the most abundant cDNA was held constant at 0.6 µg/dish and the other cDNA was reduced by a factor of 10 or 100. Responses are to 100 µM GABA. Number of observations are shown in parenthesis.

Figures 15A-15B. Figure 15A: Agonist concentration-effect curves for 3-APMPA in oocytes (open triangle), GABA in oocytes (open circle) and HEK293 cells (solid circle), and baclofen in oocytes (open square). Figure 15B: Right-ward shifts in the GABA concentration-response curve (solid circle) caused by CGP55845 at 50 nM (open triangle) and CGP54626 at 5 μM (open circle). Each point is the average response from 4-6 oocytes.

Figure 16. Microphysiometric response to baclofen (100 μM) from CHO cells expressing combinations of $GABA_BR1$ and $GABA_BR2$ (n = 4).

Figures 17A-17D. Co-localization of $GABA_BR1$ and $GABA_BR2$ in HEK293 cells by dual wavelength scanning confocal microscopy. Figure 17A: Green channel showing $GABA_BR1^{RGS6xH}$ (labeled with FITC) in cell expressing both $GABA_BR1^{RGS6xH}$ and $GABA_BR2^{HA}$. Figure 17B: Red channel showing $GABA_BR2^{HA}$ (labeled with TRITC) localization in the same cell. Figure 17C: Dual channel image of the same cell reveals a predominant yellow hue caused by the co-localization of fluorescent tags for $GABA_BR1^{RGS6xH}$ and $GABA_BR2^{HA}$. Figure 17D: Dual wavelength image of cell expressing $GABA_BR2^{HA}$ (red) and NPY $Y5^{Flag}$ (green). Note the low degree of spatial overlap of the two polypeptides.

Figures 18A-18C. Identification of $GABA_BR1$ and $GABA_BR2$ in cell lysates and immunoprecipitates. Figure 18A: Detection of $GABA_BR1^{RGS6xH}$ in whole cell extracts from cells expressing either or both polypeptides. Proteins labeled with anti-His or anti-HA, migrate as monomeric and dimeric forms. Figure 18B: Detection of $GABA_BR2^{HA}$ in whole cell extracts from cells expressing either or both. Labels over lanes denote which polypeptides were transfected. Proteins labeled with anti-His or anti-HA, migrate as monomeric and dimeric forms. Figure 18C: Co-immunoprecipitation of $GABA_BR1^{RGS6xH}$ and $GABA_BR2^{HA}$. Variously transfected cells were immunoprecipitated (IP) with anti-HA or anti-His antibodies, subjected to SDS-PAGE, blotted, and probed for the presence of the HA epitope. Note that in anti-His immunoprecipitated material, HA immunoreactivity appears only in the lane from cells expressing both proteins.

Figure 19. Rostro-caudal distribution of the $GABA_BR2$ mRNA in coronal rat brain sections(A-F)and spinal cord (G). Brightfield photomicrographs of the dorsal root (H) and trigeminal (I) ganglia showing silver grains over the cells indicating the presence of $GABA_BR2$ mRNA.

Figure 20. (A) Detection of Na+/K+ ATPase by anti-alpha 1 subunit antibodies in membrane fractions enriched in (P1+) or depleted of (P2) plasma membranes (50 :g protein/lane). (B) Co-immunoprecipitation of $GABA_BR1^{RGS6xH}$ and $GABA_BR2^{HA}$ from solubilized P1+ membrane fractions. Note that in anti-His immunoprecipitated material, HA immunoreactivity appears only in the lane from cells expressing both proteins. (C) Western blot showing enrichment of $GABA_BR2^{HA}$ in P1+ membrane fraction as compared to the P2 fraction.

Figure 21. Photomicrographs showing the regional distribution of $GABA_BR2$ (A,C) and $GABA_BR1b$ (B,D) mRNAs in pairs of adjacent

coronal rat brain sections. Arrow heads identify Purkinje cell layer in cerebellum (A,B). High magnification views of hippocampal CA3 region showing both transcripts in cells from alternate sections (C,D). Arrows mark individual cells. Hybridization of $GABA_BR2$ (E) and $GABA_BR1b$ (F) transcripts in large cells of mesencephalic trigeminal nucleus.

DETAILED DESCRIPTION OF THE INVENTION

In this application, the following standard abbreviations are used to indicate specific nucleotide bases:

C = cytosine	A = adenine
T = thymine	G = guanine

In this application, the term 7-TM spanning protein or a 7-TM protein indicates a protein presumed to have seven transmembrane regions which cross the cellular membrane band on its amino acid sequence.

This invention is directed to an isolated nucleic acid encoding a $GABA_BR2$ polypeptide.

In one embodiment, the nucleic acid is DNA. In another embodiment, the DNA is cDNA. In another embodiment, the DNA is genomic DNA. In another embodiment, the nucleic acid is RNA. In another embodiment, the nucleic acid encodes a mammalian $GABA_BR2$ polypeptide. In another embodiment, the nucleic acid encodes a rat $GABA_BR2$ polypeptide. In another embodiment, the nucleic acid encodes a human $GABA_BR2$ polypeptide.

In another embodiment, the nucleic acid encodes a polypeptide characterized by an amino acid sequence in the transmembrane regions which has an identity of 90% or higher to the amino acid sequence in the transmembrane regions of the human $GABA_BR2$ polypeptide shown in Figures 5A-5D.

In another embodiment, the nucleic acid encodes a mammalian $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as does the $GABA_BR2$ polypeptide encoded by the plasmid BO-55 (ATCC Accession No. 209104). In another embodiment, the nucleic acid encodes a rat $GABA_BR2$ polypeptide which has an

amino acid sequence encoded by the plasmid BO-55 (ATCC Accession No. 209104).

In another embodiment, the nucleic acid encodes a rat $GABA_BR2$ polypeptide having substantially the same amino acid sequence as the amino acid sequence shown in Figures 4A-4D (Seq. ID No. 4). In another embodiment, the nucleic acid encodes a rat $GABA_BR2$ polypeptide having the amino acid sequence shown in Figures 4A-4D (Seq. ID No. 4).

In another embodiment, the nucleic acid encodes a mammalian $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as does the $GABA_BR2$ polypeptide encoded by the plasmid TL-267 (ATCC Accession No. 209103). In another embodiment, the nucleic acid encodes a human $GABA_BR2$ polypeptide which has an amino acid sequence encoded by the plasmid TL-267(ATCC Accession No. 209103).

In another embodiment, the human $GABA_BR2$ polypeptide has a sequence, which sequence comprises substantially the same amino acid sequence as the sequence shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

In another embodiment, the human $GABA_BR2$ polypeptide has a sequence, which sequence comprises the sequence shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

This application further supports an isolated nucleic acid encoding a $GABA_BR2$ polypeptide, the amino acid sequence of which is encoded by the nucleotide sequence set forth in either the Figures 1A-1E and 3A-3D.

Further, the human $GABA_BR2$ polypeptide described herein exhibits 38% amino acid identity with the $GABA_BR1a$ polypeptide, while the rat $GABA_BR2$ polypeptide described herein exhibits 98% identity with the human $GABA_BR2$ polypeptide.

The ATG encoding the methionine at position 16 is surrounded by flanking sequences which correspond to the well-known Kozak consensus sequence for translation initiation (Kozak, 1989 and Kozak, 1991), thus the sequence from amino acid 16 through amino acid 898 is believed to be the most likely polypeptide expressed by the nucleic acid. Neither the ATG encoding methionine 1 nor the ATG encoding methionine 19 has the Kozak flanking sequences; however, it is to be understood that the present invention provides a $GABA_BR2$ polypeptide having any one of the three possible starting methionines.

This invention provides a splice variant of the polypeptides disclosed herein. This invention further provides for alternate translation initiation sites and alternately spliced or edited variants of nucleic acids encoding rat and human polypeptides of this invention.

Methods for production and manipulation of nucleic acid molecules are well known in the art.

This invention also encompasses DNAs and cDNAs which encode amino acid sequences which differ from those of the polypeptides of this invention, but which should not produce phenotypic changes. Alternatively, this invention also encompasses DNAs, cDNAs, and RNAs which hybridize to the DNA, cDNA, and RNA of the subject invention. Hybridization methods are well known to those of skill in the art.

The nucleic acids of the subject invention also include nucleic acid molecules coding for polypeptide analogs, fragments or derivatives of antigenic polypeptides which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues

specified are replaced by other residues and addition analogs where in one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of naturally-occurring forms. These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors.

The modified polypeptides of this invention may be transfected into cells either transiently or stably using methods well-known in the art, examples of which are disclosed herein.

This invention also provides for binding assays using the modified polypeptides, in which the polypeptide is expressed either transiently or in stable cell lines. This invention further provides for a compound identified using a modified polypeptide in a binding assay such as the binding assays described herein.

The nucleic acids described and claimed herein are useful for the information which they provide concerning the amino acid sequence of the polypeptide and as products for the large scale synthesis of the polypeptide by a variety of recombinant techniques. The nucleic acid molecule is useful for generating new cloning and expression vectors, transformed and transfected prokaryotic and eukaryotic host cells, and new and useful methods for cultured growth of such host cells capable of expression of the polypeptide and related products.

Vectors which comprise the isolated nucleic acid molecule described hereinabove also are provided. Suitable vectors comprise, but are not limited to, a plasmid or a virus. These

vectors may be transformed into a suitable host cell to form a host cell expression system for the production of a $GABA_BR2$ polypeptide. Suitable host cells include, for example, neuronal cells such as the glial cell line C6, a Xenopus cell such as an oocyte or melanophore cell, as well as numerous mammalian cells and non-neuronal cells.

This invention further provides for any vector or plasmid which comprises modified untranslated sequences, which are beneficial for expression in desired host cells or for use in binding or functional assays. For example, a vector or plasmid with untranslated sequences of varying lengths may express differing amounts of the polypeptide depending upon the host cell used. In an embodiment, the vector or plasmid comprises the coding sequence of the polypeptide and the regulatory elements necessary for expression in the host cell.

As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. The term "complementary" is used in its usual sense in the art, i.e., G and C are complementary and A is complementary to T (or U in RNA), such that two strands of nucleic acid are "complementary" only if every base matches the opposing base exactly.

This invention is directed to a purified $GABA_BR2$ protein.

This invention is directed to a vector comprising a above-identified nucleic acid.

In one embodiment, the vector is adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the nucleic acid in the bacterial cell operatively linked to the nucleic acid encoding a $GABA_BR2$

polypeptide so as to permit expression thereof.

In another embodiment, the vector is adapted for expression in an amphibian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the amphibian cell operatively linked to the nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

In another embodiment, the vector is adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the nucleic acid in the yeast cell operatively linked to the nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

In another embodiment, the vector is adapted for expression in an insect cell which comprises the regulatory elements necessary for expression of the nucleic acid in the insect cell operatively linked to the nucleic acid encoding the $GABA_BR2$ polypeptide so as to permit expression thereof.

In one embodiment, the vector is a baculovirus.

In another embodiment, the vector is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

In one embodiment, the vector is a plasmid.

In a further embodiment, the plasmid is designated BO-55 (ATCC Accession No. 209104).

In a further embodiment, the plasmid is designated TL-267(ATCC Accession No. 209103).

This invention provides a plasmid designated TL-267 (ATCC

Accession No. 209103) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the human polypeptide so as to permit expression thereof.

This plasmid (TL-267) was deposited on June 10, 1997, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 209103.

This invention provides a plasmid designated BO-55 (ATCC Accession No. 209104) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the rat polypeptide so as to permit expression thereof.

This plasmid (BO-55) was deposited on June 10, 1997, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 209104.

Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. DNA probe molecules may be produced by insertion of a DNA molecule which encodes the polypeptides of this invention into suitable vectors, such as plasmids or bacteriophages, followed by transforming into suitable bacterial host cells, replication in the transformed bacterial host cells and harvesting of the DNA probes, using methods well known in the art. Alternatively, probes may be

generated chemically from DNA synthesizers.

RNA probes may be generated by inserting the DNA molecule which encodes the polypeptides of this invention downstream of a bacteriophage promoter such as T3, T7 or SP6. Large amounts of RNA probe may be produced by incubating the labeled nucleotides with the linearized fragment where it contains an upstream promoter in the presence of the appropriate RNA polymerase.

This invention is directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within one of the two strands of the nucleic acid encoding the $GABA_BR2$ polypeptide contained in plasmid BO-55, and detecting hybridization of the probe to the nucleic acid.

This invention is directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within (a) the nucleic acid sequence shown in Figures 1A-1E (Seq. ID No. 1) or (b) the reverse complement to the nucleic acid sequence shown in Figures 1A-1E (Seq. ID No. 1), and detecting hybridization of the probe to the nucleic acid.

This invention is directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within

one of the two strands of the nucleic acid encoding the $GABA_BR2$ polypeptide contained in plasmid TL-267, and detecting hybridization of the probe to the nucleic acid.

This invention is directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within (a) the nucleic acid sequence shown in Figures 3A-3D (Seq. ID No. 3) or (b) the reverse complement to the nucleic acid sequence shown in Figures 3A-3D (Seq. ID No. 3), and detecting hybridization of the probe to the nucleic acid.

In one embodiment, the nucleic acid is DNA.

In another embodiment, the nucleic acid is RNA.

In one embodiment, the probe comprises at least 15 nucleotides complementary to a unique segment of the sequence of the nucleic acid molecule encoding the $GABA_BR2$ polypeptide.

This invention is directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising a nucleic acid of at least 15 nucleotides which is complementary to the antisense sequence of a unique segment of the sequence of the nucleic acid encoding the $GABA_BR2$ polypeptide, and detecting hybridization of the probe to the nucleic acid.

This invention is directed to a method of inhibiting translation of mRNA encoding a $GABA_BR2$ polypeptide which comprises contacting such mRNA with an antisense oligonucleotide having a sequence capable of specifically hybridizing to the above-identified mRNA, so as to prevent translation of the mRNA.

This invention is directed to a method of inhibiting translation of mRNA encoding a $GABA_BR2$ polypeptide which comprises contacting such mRNA with an antisense oligonucleotide having a sequence capable of specifically hybridizing to the above-identified genomic DNA.

In one embodiment, the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.

In another embodiment, the isolated antibody is capable of binding to a $GABA_BR2$ polypeptide encoded by an above-identified nucleic acid.

In another embodiment, the $GABA_BR2$ polypeptide is a human $GABA_BR2$ polypeptide.

This invention is directed to an antibody capable of competitively inhibiting the binding of an above-identified antibody to a $GABA_BR2$ polypeptide.

In one embodiment, the antibody is a monoclonal antibody.

In one embodiment, the monoclonal antibody is directed to an epitope of a $GABA_BR2$ polypeptide present on the surface of a $GABA_BR2$ polypeptide expressing cell.

In another embodiment, the oligonucleotide is coupled to a substance which inactivates mRNA.

In another embodiment, the substance which inactivates mRNA is a ribozyme.

This invention is directed to a pharmaceutical composition which comprises an amount of an above-identified antibody effective to block binding of a ligand to the $GABA_BR2$ polypeptide and a pharmaceutically acceptable carrier.

This invention is directed to an above-identified transgenic, nonhuman mammal, wherein the DNA encoding the $GABA_BR2$ polypeptide additionally comprises an inducible promoter.

This invention is directed to an above-identified transgenic, nonhuman mammal, wherein the DNA encoding the $GABA_BR2$ polypeptide additionally comprises tissue specific regulatory elements.

This invention is directed to an above-identified transgenic, nonhuman mammal, wherein the transgenic, nonhuman mammal is a mouse.

This invention is directed to method of detecting the presence of a $GABA_BR2$ polypeptide on the surface of a cell which comprises contacting the cell with an above-identified antibody under conditions permitting binding of the antibody to the polypeptide, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a $GABA_BR2$ polypeptide on the surface of the cell.

This invention is directed to a method of preparing a purified $GABA_BR2$ polypeptide which comprises:

a. inducing cells to express a $GABA_BR2$ polypeptide;

b. recovering the polypeptide so expressed from the induced cells; and

c. purifying the polypeptide so recovered.

This invention is directed to a method of preparing the purified $GABA_BR2$ polypeptide which comprises:

a. inserting a nucleic acid encoding the $GABA_BR2$ polypeptide into a suitable vector;

b. introducing the resulting vector in a suitable host cell;

c. placing the resulting cell in suitable condition permitting the production of the $GABA_BR2$ polypeptide;

d. recovering the polypeptide produced by the resulting cell; and

e. isolating or purifying the polypeptide so recovered.

This invention is directed to a $GABA_BR1/R2$ receptor comprising two polypeptides, one of which is a $GABA_BR2$ polypeptide and another of which is a $GABA_BR1$ polypeptide.

This invention is directed to a method of forming a $GABA_BR1/R2$ receptor which comprises inducing cells to express both a $GABA_BR1$ polypeptide and a $GABA_BR2$ polypeptide.

$GABA_BR1$ as used in this application could be $GABA_BR1a$ or $GABA_BR1b$. The observation that at least two variants of the $GABA_BR1$ polypeptide exist raises the possibility that $GABA_BR2$ splice variants may exist or that there may exist introns in coding or non-coding regions of the genes encoding the $GABA_BR2$ polypeptides. In addition, spliced form(s) of mRNA may encode additional amino acids either upstream of the currently defined starting methionine or within the coding region. Further, the existence and use of alternative exons is possible, whereby the mRNA may encode different amino acids within the region comprising the exon. In addition, single amino acid substitutions may arise via the mechanism of RNA editing such that the amino acid sequence of the expressed protein is different than that encoded by the original gene (Burns et al., 1996; Chu et al., 1996). Such variants may exhibit pharmacologic properties differing from the polypeptide encoded by the original gene.

The activity of a G-protein coupled receptor (GPCR) typically is measured using any of a variety of functional assays in which activation of the receptor in question results in an observable change in the level of some second messenger system, including but not limited to adenylate cyclase, calcium mobilization, arachidonic acid release, ion channel activity, inositol phospholipid hydrolysis or guanylyl cyclase. Heterologous expression systems utilizing appropriate host cells to express the nucleic acids of the subject invention are used to obtain the desired second messenger coupling. Receptor activity may also be assayed in an oocyte expression system.

The pharmacologic properties of the receptor described herein when $GABA_BR2$ is co-expressed with $GABA_BR1$, are similar to the pharmacologic properties of the $GABA_B$ receptor observed using tissues. For convenience, in the context of the present invention applicants will refer to the product of the heterologous coexpression of $GABA_BR2$ and $GABA_BR1$ as the "$GABA_BR1/R2$ receptor." Thus, a cell expressing nucleic acid encoding a $GABA_BR1/R2$ receptor is to be understood to refer to a cell expressing both nucleic acid encoding a $GABA_BR1$ polypeptide and nucleic acid encoding a $GABA_BR2$ polypeptide. In this application, $GABA_BR1$ can be either $GABA_BR1a$ or $GABA_BR1b$.

This invention is directed to an antibody capable of binding to a $GABA_BR1/R2$ receptor, wherein the $GABA_BR2$ polypeptide is encoded by an above-identified nucleic acid.

This invention is directed to an above-identified antibody, wherein the $GABA_BR2$ polypeptide is a human $GABA_BR2$ polypeptide.

This invention is directed to an antibody capable of competitively inhibiting the binding of an above-identified antibody to a $GABA_BR1/R2$ receptor.

In one embodiment, the antibody is a monoclonal antibody.

This invention is directed to an above-identified monoclonal antibody directed to an epitope of a $GABA_B$R1/R2 receptor present on the surface of a $GABA_B$R1/R2 polypeptide expressing cell.

This invention is directed to a pharmaceutical composition which comprises an amount of an above-identified antibody effective to block binding of a ligand to the $GABA_B$R1/R2 receptor and a pharmaceutically acceptable carrier.

This invention is directed to a transgenic, nonhuman mammal expressing a $GABA_B$R1/R2 receptor, which is not naturally expressed by the mammal.

This invention is directed to a transgenic, nonhuman mammal comprising a homologous recombination knockout of the native $GABA_B$R1/R2 receptor.

In one embodiment, the transgenic nonhuman mammal is a mouse.

This invention is directed to a method of detecting the presence of a $GABA_B$R1/R2 receptor on the surface of a cell which comprises contacting the cell with an above-identified antibody under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a $GABA_B$R1/R2 receptor on the surface of the cell.

This invention is directed to a method of determining the physiological effects of varying levels of activity of $GABA_B$R1/R2 receptors which comprises producing an above-identified transgenic nonhuman mammal whose levels of $GABA_B$R1/R2 receptor activity vary due to the presence of an inducible promoter which regulates $GABA_B$R1/R2 receptor expression.

This invention is directed to a method of determining the physiological effects of varying levels of activity of $GABA_BR1/R2$ receptors which comprises producing a panel of above-identified transgenic nonhuman mammals, each expressing a different amount of $GABA_BR1/R2$ receptor.

This invention is directed to a method for identifying an antagonist capable of alleviating an abnormality, by decreasing the activity of a $GABA_BR1/R2$ receptor comprising administering a compound to a above-identified transgenic nonhuman mammal, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as the antagonist.

This invention is directed to an antagonist identified by an above-identified method.

This invention is directed to a pharmaceutical composition comprising an above-identified antagonist and a pharmaceutically acceptable carrier.

This invention is directed to a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a $GABA_BR1/R2$ receptor which comprises administering to a subject an effective amount of an above-identified pharmaceutical composition, thereby treating the abnormality.

This invention is directed to a method for identifying an agonist capable of alleviating an abnormality, by increasing the activity of a $GABA_BR1/R2$ receptor comprising administering a compound to an above-identified transgenic nonhuman mammal, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as the agonist.

This invention is directed to an agonist identified by an above-identified method.

This invention is directed to a pharmaceutical composition comprising an above-identified agonist and a pharmaceutically acceptable carrier.

This invention is directed to a method for treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a $GABA_BR1/R2$ receptor which comprises administering to a subject an effective amount of an above-identified pharmaceutical composition, thereby treating the abnormality.

This invention is directed to a cell which expresses on its surface a mammalian $GABA_BR1/R2$ receptor that is not naturally expressed on the surface of such cell.

This invention is directed to a cell, wherein the mammalian $GABA_BR1/R2$ receptor comprises two polypeptides, one of which is a $GABA_BR2$ polypeptide and another of which is a $GABA_BR1$ polypeptide.

This invention is directed to a process for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor.

This invention is directed to a process for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises contacting a membrane fraction from a cell extract of cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor,

wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor.

In one embodiment, the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same sequence as the amino acid sequence shown in Figures 2A-2D (Seq. ID No. 2).

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the amino acid sequence shown in Figures 2A-2D (Seq. ID No. 2).

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid TL-267 (ATCC Accession No. 209103).

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as the sequence shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the sequence shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

In another embodiment, the compound is not previously known to bind to a $GABA_BR1/R2$ receptor.

This invention is directed to a compound identified by an above-identified process.

In one embodiment, the cell is an insect cell.

In another embodiment, the cell is a mammalian cell.

In another embodiment, the cell is nonneuronal in origin.

In another embodiment, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell a mouse Y1 cell or LM(tk-) cell.

In another embodiment, the compound is not previously known to bind to a $GABA_BR1/R2$ receptor.

This invention is directed to a compound identified by an above-identified process.

This invention is directed to a process involving competitive binding for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises separately contacting cells expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor, a decrease in the binding of the second chemical compound to the $GABA_BR1/R2$ receptor in the presence of the chemical compound indicating that the chemical compound binds to the $GABA_BR1/R2$ receptor.

This invention is directed to a process involving competitive binding for identifying a chemical compound which specifically binds to a human $GABA_BR1/R2$ receptor which comprises separately contacting a membrane fraction from a cell extract of cells expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor, a decrease in the binding of the second chemical compound to the $GABA_BR1/R2$ receptor in the presence of the chemical compound indicating that the chemical compound binds to the $GABA_BR1/R2$ receptor.

In one embodiment, the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by plasmid BO-55 (ATCC Accession No. 209104).

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in Figures 2A-2D (Seq. ID No. 2).

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the amino acid sequence shown in Figures 2A-2D (Seq. ID No. 2).

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by plasmid TL-267 (ATCC Accession No. 209103).

In another embodiment, the $GABA_BR1/R2$ receptor comprises a

$GABA_BR2$ polypeptide which has substantially the same amino acid sequence as the sequence shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the sequence shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

In another embodiment, the cell is an insect cell.

In another embodiment, the cell is a mammalian cell..

In another embodiment, the cell is nonneuronal in origin.

In another embodiment, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell a mouse Y1 cell or LM(tk-) cell.

In another embodiment, the compound is not previously known to bind to a $GABA_BR1/R2$ receptor.

This invention is directed to a compound identified by an above-identified process.

This invention is directed to a method of screening a plurality of chemical compounds not known to bind to a $GABA_BR1/R2$ receptor to identify a compound which specifically binds to the $GABA_BR1/R2$ receptor, which comprises

(a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with a compound known to bind specifically to the $GABA_BR1/R2$ receptor;

(b) contacting the same cells as in step (a) with the plurality of compounds not known to bind

specifically to the $GABA_BR1/R2$ receptor, under conditions permitting binding of compounds known to bind the $GABA_BR1/R2$ receptor;

(c) determining whether the binding of the compound known to bind specifically to the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of the compounds, relative to the binding of the compound in the absence of the plurality of compounds, and if the binding is reduced;

(d) separately determining the extent of binding to the $GABA_BR1/R2$ receptor of each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which specifically binds to the $GABA_BR1/R2$ receptor.

This invention is directed to a method of screening a plurality of chemical compounds not known to bind to a $GABA_BR1/R2$ receptor to identify a compound which specifically binds to the $GABA_BR1/R2$ receptor, which comprises

(a) contacting a membrane fraction extract from cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with a compound known to bind specifically to the $GABA_BR1/R2$ receptor;

(b) contacting the same membrane fraction as in step (a) with the plurality of compounds not known to bind specifically to the $GABA_BR1/R2$ receptor, under conditions permitting binding of compounds known to bind the $GABA_BR1/R2$ receptor;

(c) determining whether the binding of the compound

known to bind specifically to the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds, and if the binding is reduced;

(d) separately determining the extent of binding to the $GABA_BR1/R2$ receptor of each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which specifically binds to the $GABA_BR1/R2$ receptor.

In one embodiment, the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

In one embodiment, the cell is a mammalian cell.

In one embodiment, the mammalian cell is non-neuronal in origin.

In one embodiment, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell or an NIH-3T3 cell.

This invention is directed to a process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor agonist which comprises contacting cells with the compound under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting an increase in $GABA_BR1/R2$ receptor activity, so as to thereby determine whether the compound is a $GABA_BR1/R2$ receptor agonist.

This invention is directed to a process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor antagonist which comprises contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$

receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the compound in the presence of a known $GABA_BR1/R2$ receptor agonist, under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting a decrease in $GABA_BR1/R2$ receptor activity, so as to thereby determine whether the compound is a $GABA_BR1/R2$ receptor antagonist.

Expression of genes in Xenopus oocytes is well known in the art (A. Coleman, Transcription and Translation: A Practical Approach (B.D. Hanes, S.J. Higgins, eds., pp 271-302, IRL Press, Oxford, 1984; Y. Masu et al., Nature **329**:21583-21586, 1994) and is performed using microinjection of native mRNA or *in vitro* synthesized mRNA into frog oocytes. The preparation of *in vitro* synthesized mRNA can be performed by various standard techniques (J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989) including using T7 polymerase with the mCAP RNA capping kit (Stratagene).

In one embodiment, the cells additionally express nucleic acid encoding GIRK1 and GIRK4.

In another embodiment, the $GABA_BR2$ receptor is a mammalian $GABA_BR2$ receptor.

This invention is directd to a pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor agonist determined to be an agonist by an above-identified process effective to increase activity of a $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

This invention is directed to a pharmaceutical, wherein the $GABA_BR1/R2$ receptor agonist was not previously known.

This invention is directed to a pharmaceutical composition which comprises an amount of a $GABA_B$R1/R2 receptor antagonist determined to be an antagonist an above-identified process effective to reduce activity of a $GABA_B$R1/R2 receptor and a pharmaceutically acceptable carrier.

This invention is directed to a pharmaceutical composition, wherein the $GABA_B$R1/R2 receptor antagonist was not previously known.

This invention is directed to a process for determining whether a chemical compound activates a $GABA_B$R1/R2 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the $GABA_B$R1/R2 receptor, wherein such cells do not normally express the $GABA_B$R1/R2 receptor, with the chemical compound under conditions suitable for activation of the $GABA_B$R1/R2 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the $GABA_B$R1/R2 receptor.

In one embodiment, the second messenger response comprises potassium channel activation and the change in second messenger is an increase in the level of potassium current.

This invention is directed to a process for determining whether a chemical compound inhibits activation of a $GABA_B$R1/R2 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the $GABA_B$R1/R2 receptor, wherein such cells do not normally express the $GABA_B$R1/R2 receptor, with both the chemical compound and a second chemical compound known to activate the $GABA_B$R1/R2 receptor, and with only the second chemical compound, under conditions suitable for activation of the $GABA_B$R1/R2 receptor, and measuring the second messenger

response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the $GABA_BR1/R2$ receptor.

In one embodiment, the second messenger response comprises potassium channel activation and the change in second messenger response is a smaller increase in the level of inward potassium current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

This invention is directed to an above-identified process, wherein the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

In one embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in Figures 4A-4D (Seq. ID No. 4).

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the sequence, shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid

898.

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid TL-267 (ATCC Accession No. 209103).

This invention is directed to an above-identified process, wherein the cell is an insect cell.

This invention is directed to an above-identified process, wherein the cell is a mammalian cell.

In one embodiment, the mammalian cell is nonneuronal in origin.

In another embodiment, the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

In another embodiment, the compound was not previously known to activate or inhibit a $GABA_BR1/R2$ receptor.

This invention is directed to a compound determined by an above-identified process.

This invention is directed to a pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor agonist determined by an above-identified process effective to increase activity of a $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

In one embodiment, the $GABA_BR1/R2$ receptor agonist was not previously known.

This invention is directed to a pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor antagonist

determined by an above-identified process effective to reduce activity of a $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

In one embodiment, the $GABA_BR1/R2$ receptor antagonist was not previously known.

This invention is directed to method of screening a plurality of chemical compounds not known to activate a $GABA_BR1/R2$ receptor to identify a compound which activates the $GABA_BR1/R2$ receptor which comprises:

(a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the plurality of compounds not known to activate the $GABA_BR1/R2$ receptor, under conditions permitting activation of the $GABA_BR1/R2$ receptor;

(b) determining whether the activity of the $GABA_BR1/R2$ receptor is increased in the presence of the compounds, and if it is increased;

(c) separately determining whether the activation of the $GABA_BR1/R2$ receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which activates the $GABA_BR1/R2$ receptor.

In one embodiment, the cells express nucleic acid encoding GIRK1 and GIRK4.

In another embodiment, the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

This invention is directed to a method of screening a plurality of chemical compounds not known to inhibit the activation of a $GABA_BR1/R2$ receptor to identify a compound which inhibits the activation of the $GABA_BR1/R2$ receptor, which comprises:

(a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the plurality of compounds in the presence of a known $GABA_BR1/R2$ receptor agonist, under conditions permitting activation of the $GABA_BR1/R2$ receptor;

(b) determining whether the activation of the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of compounds, relative to the activation of the $GABA_BR1/R2$ receptor in the absence of the plurality of compounds, and if it is reduced;

(c) separately determining the inhibition of activation of the $GABA_BR1/R2$ receptor for each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such a plurality of compounds which inhibits the activation of the $GABA_BR1/R2$ receptor.

In one embodiment, the cells express nucleic acid encoding GIRK1 and GIRK4.

In one embodiment, the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

In another embodiment, wherein the cell is a mammalian cell.

In another embodiment, the mammalian cell is non-neuronal in origin.

In another embodiment, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell or an NIH-3T3 cell.

This invention is directed to a pharmaceutical composition comprising a compound identified by an above-identified method, effective to increase $GABA_B$R1/R2 receptor activity and a pharmaceutically acceptable carrier.

This invention is directed to a pharmaceutical composition comprising a compound identified by an above-identified method, effective to decrease $GABA_B$R1/R2 receptor activity and a pharmaceutically acceptable carrier.

This invention is directed to a process for determining whether a chemical compound is a $GABA_B$R1/R2 receptor agonist, which comprises preparing a membrane fraction from cells which comprise nucleic acid encoding and expressing on their cell surface the $GABA_B$R1/R2 receptor, wherein such cells do not normally express the $GABA_B$R1/R2 receptor, separately contacting the membrane fraction with both the chemical compound and GTPγS, and with only GTPγS, under conditions permitting the activation of the $GABA_B$R1/R2 receptor, and detecting GTPγS binding to the membrane fraction, an increase in GTPγS binding in the presence of the compound indicating that the chemical compound activates the $GABA_B$R1/R2 receptor.

This invention is directed to a process for determining whether a chemical compound is a $GABA_B$R1/R2 receptor antagonist, which comprises preparing a membrane fraction from cells which comprise nucleic acid encoding and expressing on their cell surface the $GABA_B$R1/R2 receptor, wherein such cells do not normally express the $GABA_B$R1/R2 receptor, separately contacting the membrane fraction with the chemical compound, GTPγS and a second chemical compound known to activate the $GABA_B$R1/R2 receptor, with GTPγS and only the second compound, and with GTPγS alone, under conditions permitting the

activation of the $GABA_BR1/R2$ receptor, detecting GTPγS binding to each membrane fraction, and comparing the increase in GTPγS binding in the presence of the compound and the second compound relative to the binding of GTPγS alone, to the increase in GTPγS binding in the presence of the second chemical compound known to activate the $GABA_BR1/R2$ receptor relative to the binding of GTPγS alone, a smaller increase in GTPγS binding in the presence of the compound and the second compound indicating that the compound is a $GABA_BR1/R2$ receptor antagonist.

In one embodiment, the $GABA_BR2$ receptor is a mammalian $GABA_BR2$ receptor.

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in Figures 4A-4D (Seq. ID No. 4).

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid TL-267 (ATCC Accession No. 209103).

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the sequence shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

In another embodiment, the cell is an insect cell.

In another embodiment, the cell is a mammalian cell.

In another embodiment, the mammalian cell is nonneuronal in origin.

In another embodiment, the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

In another embodiment, the compound was not previously known to be an agonist or antagonist of a $GABA_B$R1/R2 receptor.

This invention is directed to a compound determined to be an agonist or antagonist of a $GABA_B$R1/R2 receptor by an above-identified process.

This invention is directed to a method of treating spasticity in a subject which comprises administering to the subject an amount of a compound which is an agonist of a $GABA_B$R1/R2 receptor effective to treat spasticity in the subject.

This invention is directed to a method of treating asthma in a subject which comprises administering to the subject an amount of a compound which is a $GABA_B$R1/R2 receptor agonist effective to treat asthma in the subject.

This invention is directed to a method of treating incontinence in a subject which comprises administering to the subject an amount of a compound which is a $GABA_B$R1/R2 receptor agonist effective to treat incontinence in the subject.

This invention is directed to method of decreasing nociception in a subject which comprises administering to the subject an amount of a compound which is a $GABA_B$R1/R2 receptor agonist effective to decrease nociception in the subject.

This invention is directed to a use of a $GABA_BR2$ agonist as an antitussive agent which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective as an antitussive agent in the subject.

This invention is directed to a method of treating drug addiction in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to treat drug addiction in the subject.

This invention directed to a method of treating Alzheimer's disease in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor antagonist effective to treat Alzheimer's disease in the subject.

This invention is directed to a peptide selected from the group consisting of:

a) P L Y S I L S A L T I L G M I M A S A F L F F N I K N;
b) L I I L G G M L S Y A S I F L F G L D G S F V S E K T;
c) C T V R T W I L T V G Y T T A F G A M F A K T W R;
d) Q K L L V I V G G M L L I D L C I L I C W Q;
e) M T I W L G I V Y A Y K G L L M L F G C F L A W;
f) A L N D S K Y I G M S V Y N V G I M C I I G A A V; and
g) C I V A L V I I F C S T I T L C L V F V P K L I T L R T N .

This invention is directed to a compound that prevents the formation of a $GABA_BR1/R2$ receptor complex.

Transmembrane peptides derived from $GABA_BR2$ sequences may modulate the functional activity of $GABA_BR1/R2$ receptors. One mode of action involves the destruction of the $GABA_BR1/R2$ receptor complex *via* competitive displacement of the $GABA_BR2$

polypeptide subunit by the peptide upon binding to the $GABA_BR1$ polypeptide subunit. The peptides may be synthesized using standard solid phase F-moc peptide synthesis protocol using an Advanced Chemtech 396 Automated Peptide Synthesizer.

Additional $GABA_B$ subtypes in hypothalamus and caudate putamen are predicted due to the under-representation of $GABA_BR2$ hybridization signals. These novel $GABA_B$ proteins and others may be identified by using $GABA_BR2$ polypeptides in co-immunoprecipitation experiments.

This invention provides a process for making a composition of matter which specifically binds to a $GABA_BR1/R2$ receptor which comprises identifying a chemical compound using any of the processes descirbed herein for identifying a compound which binds to and/or activates or inhibits activation of a $GABA_BR1/R2$ receptor and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof. In one embodiment, the $GABA_BR1/R2$ receptor is a human $GABA_BR1/R2$ receptor.

This invention further provides a process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by any of the processes described herein for identifying a compound which binds to and/or activates or inhibits activation of a $GABA_BR1/R2$ receptor or a novel structural and functional analog or homolog thereof. In one embodiment, the $GABA_BR1/R2$ receptor is a human $GABA_BR1/R2$ receptor.

Thus, once the gene for a targeted receptor subtype is cloned, it is placed into a recipient cell which then expressses the targeted receptor subtype on its surface. This cell, which expresses a single population of the targeted human receptor

subtype, is then propagated resulting in the establishment of a cell line. This cell line, which constitutes a drug discovery system, is used in two different types of assays: binding assays and functional assays. In binding assays, the affinity of a compound for both the receptor subtype that is the target of a particular drug discovery program and other receptor subtypes that could be associated with side effects are measured. These measurements enable one to predict the potency of a compound, as well as the degree of selectivity that the compound has for the targeted receptor subtype over other receptor subtypes. The data obtained from binding assays also enable chemists to design compounds toward or away from one or more of the relevant subtypes, as appropriate, for optimal therapeutic efficacy. In functional assays, the nature of the response of the receptor subtype to the compound is determined. Data from the functional assays show whether the compound is acting to inhibit or enhance the activity of the receptor subtype, thus enabling pharmacologists to evaluate compounds rapidly at their ultimate human receptor subtypes targets permitting chemists to rationally design drugs that will be more effective and have fewer or substantially less severe side effects than existing drugs.

Approaches to designing and synthesizing receptor subtype-selective compounds are well known and include traditional medicinal chemistry and the newer technology of combinatorial chemistry, both of which are supported by computer-assisted molecular modeling. With such approaches, chemists and pharmacologists use their knowledge of the structures of the targeted receptor subtype and compounds determined to bind and/or activate or inhibit activation of the receptor subtype to design and synthesize structures that will have activity at these receptor subtypes.

Combinatorial chemistry involves automated synthesis of a

variety of novel compounds by assembling them using different combinations of chemical building blocks. The use of combinatorial chemistry greatly accelerates the process of generating compounds. The resulting arrays of compounds are called libraries and are used to screen for compounds (lead compounds) that demonstrate a sufficient level of activity at receptors of interest. Using combinatorial chemistry it is possible to synthesize focused libraries of compounds anticiapted to be highly biased toward the receptor target of interest.

Once lead compounds are identified, whether through the use of combinatorial chemistry or traditional medicinal chemistry or otherwise, a variety of homologs and analogs are prepared to facilitate an understanding of the relationship between chemical structure and biological or functional activity. These studies define structure activity relationships which are then used to design drugs with improved potency, selectivity and pharmacokinetic properties. Combinatorial chemistry is also used to rapidly generate a variety of structures for lead optimization. Traditional medicinal chemistry, which involves the synthesis of compounds one at a time, is also used for further refinement and to generate compounds not accessible by autometed techniques. Once such drugs are defined the production is scaled up using standard chemical manufacturing methodiologies utilized throughout the pharmaceutical and chemistry industry.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

Materials and Methods

DNA Sequencing

DNA sequences were determined using an ABI PRISM 377 DNA Sequencer (Perkin-Elmer, Foster City, CA) according to the manufacturer's instructions.

Hybridization methodology

Probes were end-labeled with polynucleotide kinase according to the manufacturer's instructions (Boehringer-Mannheim). Hybridization was performed on Zeta-Probe membrane (Bio-Rad, CA) at reduced stringency: 40°C in a solution containing 25% formamide, 5x SSC (1x SSC = 0.15 M NaCl, 0.015 M sodium citrate), 1x Denhardt's solution (0.02% polyvinylpyrrolindone, 0.02% Ficoll, 0.02% bovine serum albumin) and 25 µg/µL sonicated salmon sperm DNA. Membrane strips were washed at 40°C in 0.1x SSC containing 0.1% SDS and exposed at -70°C to Kodak XAR film in the presence of an intensifying screen.

The nucleotide sequences of the hybridization probes are shown below:

T-891: 5'-AGGGATGCTTTCCTATGCTTCCATATTTCTCTTTGGCCTTGATGG-3' (Seq. ID No. 5) Nucleotides 1449-1493 of TL-267, forward strand.

T-892: 5'-CAATGTGCAGTTCTGCATCGTGGCTCTGGTCATCATCTTCTGCAG-3' (Seq. ID No. 6) Nucleotides 2022-2066 of TL-267, forward strand.

PCR Methodology

PCR reactions were carried out using a PE 9600 (Perkin-Elmer) PCR cycler in 20 µL volumes using Expand Long Template

Polymerase (Boehringer-Mannheim) and the manufacturer's buffer 1 for internal PCR primers or manufacturer's buffer 2 for vector-anchored PCR. Reactions were run using a program consisting of 35 cycles of 94°C for 30 sec., 68°C for 20 sec, and 72°C for 1 min, with a pre-incubation at 95°C for 5 min and post-incubation hold at 4°C.

Nucleotide sequences of the primer sets used in PCR reactions are shown below:

T-94: 5'-CTTCTAGGCCTGTACGGAAGTGTT-3' (Seq. ID No. 7); vector, forward primer.

T-95: 5'-GTTGTGGTTTGTCCAAACTCATCAAT-3' (Seq. ID No. 8); vector, reverse primer.

T-887: 5'-GGGATGAGTGTCTACAACGTGGGG-3' (Seq. ID No. 9); nucleotides 1948-1971 of TL-267, forward primer.

T-888: 5'-TGCGTTGCTGCATCTGGGTTTGTTCT-3' (Seq. ID No. 10); nucleotides 2138-2113 of TL-267, reverse primer.

T-889: 5'-ATCTCCCTACCTCTCTACAGCATCCT-3' (Seq. ID No. 11); nucleotides 1300-1325 of TL-267, forward primer.

T-890: 5'-CAGGTCCTGACGGTGCAAAGTGTTTC-3' (Seq. ID No. 12); nucleotides 1544-1519 of TL-267, reverse primer.

T-921: 5'-TGACGCAAGACGTTCAGAGGTTCTCT-3' (Seq. ID No. 13); nucleotides 473-498 of TL-267, forward primer.

T-922: 5'-TGTAGCCTTCCATGGCAGCAAGCAGA-3' (Seq. ID No. 14); nucleotides 814-789 of TL-267, reverse primer.

T-923: 5'-AGAGAACCTCTGAACGTCTTGCGTCA-3' (Seq. ID No. 15);

nucleotides 498-473 of TL-267, reverse primer.

T-935: 5'-GGCTCTGTTGTGTTCCACTGTAGCTG-3' (Seq. ID No. 16); nucleotides 2483-2458 of TL-267, reverse primer.

T-938: 5'-TCATGCCGCTCACCAAGGAGGTGGCC-3' (Seq. ID No. 17); nucleotides 53 to 78 of TL-267, forward primer.

T-939: 5'-GGCCACCTCCTTGGTGAGCGGCATGA-3' (Seq. ID No. 18); nucleotides 78 to 53 of TL-267, reverse primer.

T-947: 5'-TGAGTGAGCAGAGTCCAGAGCCGT-3' (Seq. ID No. 19); nucleotides -68 to -45 of TL-267, forward primer.

T-948: 5'-ATGGATGGGAGGTAGGCGTGGTGGAG-3' (Seq. ID No. 20); nucleotides 2591-2566 of TL-267, reverse primer.

<u>Preparation of human hippocampal cDNA library</u>

Total RNA was prepared by a modification of the guanidine thiocyanate method, from 6 grams of human hippocampus. Poly A^{+}RNA was purified with a FastTrack kit (Invitrogen Corp., San Diego, CA). Double stranded (ds) cDNA was synthesized from 4 µg of poly A^{+} RNA according to Gübler and Hoffman (1983), except that ligase was omitted in the second strand cDNA synthesis. The resulting DS cDNA was ligated to BstxI/EcoRI adaptors (Invitrogen Corp.), the excess of adaptors was removed by exclusion chromatography. High molecular weight fractions were ligated in pcEXV.BS (An Okayama and Berg expression vector) cut by BstxI as described by Aruffo and Seed (1987). The ligated DNA was electroporated in E. coli MC 1061 (Gene Pulser, Biorad). A total of 2.2×10^{6} independent clones with an insert mean size of approximately 3 kb was generated. The library was plated on Petri dishes (Ampicillin selection) in pools of 0.4 to 1.2×10^{4} independent clones. After 18 hours amplification, the bacteria from each pool were

scraped, resuspended in 4 mL of LB media and 1.5 mL processed for plasmid purification by the alkali method (Sambrook et al, 1989). 1 mL aliquots of each bacterial pool were stored at -85°C in 20% glycerol.

BLAST Search that Identified a Novel 7-TM protein Sequence

Sequence analysis was performed with the Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin. The rat $GABA_BR1a$ amino acid sequence (Kaupmann et al. (1997) Nature 386:239) was used as a query to search the EST division of GenBank with BLAST. Two entries, T07621 and Z43654, had probability scores that suggested significant amino acid homology to the $GABA_BR1a$ polypeptide. T07621 had sequence homology from the beginning of the first transmembrane domain to the beginning of third transmembrane domain of the $GABA_BR1a$ polypeptide. Z43654 had sequence homology from the sixth transmembrane domain to the seventh transmembrane domain of the $GABA_BR1a$ polypeptide. The sequence documentation for T07621 and Z43654 was retrieved with Entrez (NCBI) and neither sequence was annotated as having homology to any 7-TM spanning protein.

T07621 and Z43654 are part of the same sequence.

A series of PCR reactions were carried out on human hippocampus DNA with multiple primer sets: primer set T-887/T-888 designed to Z43654 sequence; primer set T-889/T-890 designed to the T07621 sequence; and primer set T-889/T-888 designed to the forward strand of T07621 and the reverse stand of Z43654. The PCR products was loaded on duplicate lanes of an agarose gel and the DNA was southern blotted to a Zeta-Probe membrane (Bio-Rad, CA). The regions of the membrane corresponding to the individual lanes on the gel were cut to produce membrane strips that contained duplicate samples of the DNA. One set of membrane strips was hybridized with T-891, a probe specific for the T07621 sequence. Another

set of membranes was hybridized with T-892, a probe specific to the Z43654 sequence. The membrane from primer set T-887/T-888 hybridized with probe T-892 for the Z43654 sequence. The membrane from primer set T-889/T-890 hybridized with probe T-891 for the T07621 sequence. The membrane from primer set T889/T-888 hybridized with both the T-891 and T-892 probes.

<u>Isolating the full-length human cDNA by PCR Sib Selection.</u> PCR reactions were carried out on bacterial pools containing a human hippocampus cDNA library. Primer set T-888/T-889 was used to identify the bacterial pools that contained a portion of the novel receptor. Vector-anchored PCR was carried out on the positive pools to determine which pool contained the longest cDNA insert. Four primer sets were used for the vector-anchored PCR: T-94/T-888, T-94/T889, T-95/T888, and T-95/T889. Pool 365 was identified having the longest cDNA inset and the plasmid was sib selected (McCormick, 1987). The nucleotide sequence of clone 365-9-7-4, designated TL-260, was translated into amino acids and compared to the amino acid sequence of the rat $GABA_BR1a$ polypeptide. Relative the rat $GABA_BR1a$ amino acid sequence, TL-260 was truncated at the amino terminus.

A set of PCR primers (T-921/T-922) was made to the 5′ region of TL-260 and was used to re-screen the bacterial pools of the human hippocampus library for the missing segment of the novel clone. Vector-anchored PCR was carried out on the positive pools to determine which pool contained the longest cDNA insert. Four primer sets were used for the vector-anchored PCR: T-94/T-921, T-94/T922, T-95/T921, and T-95/T-922. Pool 299 contained the most 5′ sequence. A PCR product derived from the primer set T-94/T-923 was isolated (T-261) and sequenced. The putative amino acids derived from TL-261 were compared to the rat $GABA_BR1$ sequence. TL-261 contained an initiation codon but didn't contain a stop codon upstream of

the initiation codon.

A set of PCR primers (T-938/T-935) was made to the 5' region of TL-261 and was used to re-screen the bacterial pools of the human hippocampus library for additional sequence. Vector-anchored PCR was carried out on the positive pools to determine which pool contained the longest cDNA insert. Four primer sets were used for the vector-anchored PCR: T-94/T-938, T-94/T939, T-95/T938, and T-95/T-939. A PCR product derived from primer set T-95/T-939 was isolated (T-261a) and sequenced. The putative amino acids derived from T-261a were compared to the rat GABA-1 amino acid sequence. T-261a contained an initiation codon and an in-frame upstream stop codon.

From the vector-anchored PCR, pool 389 contained the longest cDNA insert. This pool was sib selected with the primer set T-947/T-935. The resulting plasmid, 389-20-29-2, was designated TL-266 and was sequenced.

<u>Construction of $GABA_BR2$ polypeptide in expression vector</u>
A Cla-I-Xba-I fragment from TL-266 was subcloned into the expression vector pEXJ.HRT3T7 and designated TL-267. This plasmid was deposited with the ATCC on June 10, 1997, and was accorded ATCC Accession No. 209103.

<u>Generation of rat $GABA_BR2$ PCR product</u>
cDNA from rat hippocampus and rat cerebellum were amplified in 50μL PCR reaction mixtures using the Expand Long Template PCR System (as supplied and described by the manufacturer, Boehringer Mannheim) using a program consisting of 40 cycles of 94°C for 1 min, 50°C for 2 min, and 68°C for 2 min, with a pre- and post-incubation of 95°C for 5 min and 68°C for 7 min, respectively. PCR primers for rat $GABA_BR2$ were designed against the human $GABA_BR2$ sequence: BB 257, forward primer in

the first transmembrane domain, and BB 258, reverse primer in the seventh transmembrane domain. The single 780 bp fragment from both rat hippocampus and rat cerebellum were isolated from a 1% agarose gel, purified using a GENECLEAN III kit (BIO 101, Vista, CA) and sequenced using AmpliTaq DNA Polymerase, FS (Perkin Elmer). The sequence was run on an ABI PRISM 377 DNA Sequencer and analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, WI). This sequence was used to design PCR primers for the rat $GABA_BR2$ gene.

<u>Construction and screening of a rat hypothalamic cDNA library</u>

Poly A+ RNA was purified from rat hypothalamic RNA (Clontech) using a FastTrack kit (Invitrogen, Corp.). DS-cDNA was synthesized from 5 μg of poly A+ RNA according to Gubler and Hoffman (1983) with minor modifications. The resulting cDNA was ligated to BstXI adaptors (Invitrogen, Corp.) And the excess adapters removed by exclusion column chromatography. High molecular weight fractions of size-selected ds-cDNA were ligated in pEXJ.T7, an Okayama and Berg expression vector modified from pcEXV (Miller and Germain, 1986) to contain BstXI, other additional restriction sites, and a T7 promoter. A total of 100,000 independent clones with a mean insert size of 3.7 kb were generated. The library was amplified on agar plates (Ampicillin selection) in 48 primary pools. Glycerol stocks of the primary pools screened for a rat $GABA_BR2$ gene by PCR using BB265, a forward primer from the loop between transmembrane domains 3 and 4 from the sequence determined above and BB266, a reverse primer from the sixth transmembrane domain from the sequence determined above. The conditions for PCR were 1 min at 94°C, 4 min at 68°C for 40 cycles, with a pre- and post-incubation of 5 min at 95°C and 7 min at 68°C, respectively. To determine which pools had the largest inserts, positive pools were screened by PCR using the vector primers BB172 or BB173, and a gene-specific primer BB265 or BB266. One positive primary pool, I-47, was subdivided into

24 pools of 1000 clones, and grown in LB medium overnight. Two µL of cultures were screened by PCR using primers BB172 and BB266. One positive subpool, I-47-4 was subdivided into 10 pools of 200 clones and plated on agar plates (ampicillin selection). Colonies were transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, NH), denatured in 0.4 N NaOH, 1.5 M NaCl, renatured in 1M Tris, 1.5 M NaCl, and UV cross-linked. Filters were hybridized overnight at 40°C in a buffer containing 50 % formamide, 0.12 M Na_2HPO_4 (pH7.2), 0.25M NaCl, 7%SDS, 25 mg/L ssDNA and 10^6 cpm/mL of a cDNA probe corresponding to transmembrane domains 1 to 7 of rat $GABA_BR2$, labeled with [^{32}P]dCTP (3000Ci/mmol, NEN) using a random prime labeling kit (Boehringer Mannheim). Filters were washed 1x 5 min then 2x 20 min at room temperature in 2x SSC, 0.1%SDS then 3x 20 min at 50° in 0.1x SSC, 0.1% SDS and exposed to Biomax MS film (Kodak) for 3 hours. Four closely clustering colonies which appeared to hybridize were re-screened individually by PCR using primers BB265 and BB266, primers BB265 and BB55, primers BB265 and BB56, and primers BB266 and BB55. The conditions for PCR were 30 sec at 94°C, 2.5 min at 68°C for 32 cycles, with a pre- and post-incubation of 5 min at 95°C and 5 min at 68°C respectively. One positive colony, I-47-4-2, was amplified overnight in 10 mL TB media and processed for plasmid purification using a standard alkaline lysis miniprep procedure followed by a PEG precipitation. This plasmid was designated BO54 and partially sequenced using AmpliTaq DNA Polymerase, FS (Perkin Elmer). The sequence was run on an ABI PRISM 377 DNA Sequencer and analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, WI). BO54 was in the wrong orientation for expression in mammalian cells. To obtain a clone in the correct orientation, an *Eco*RI restriction fragment from BO54 was subcloned into the vector pEXJ. Transformants were screened by PCR using the primers BB56 and BB268 under the following conditions: 30 sec at 94°C, 2.5 min at 68°C for 32

cycles, with a pre- and post-incubation of 5 min at 95°C and 3 min at 68°C respectively. One transformant in the correct orientation was amplified overnight in 100 ml TB media and processed for plasmid purification using a standard alkaline lysis miniprep procedure followed by a PEG precipitation. This plasmid was designated BO55 and sequenced using AmpliTaq DNA Polymerase, FS (Perkin Elmer). Plasmid BO-55 was deposited with the ATCC on June 10, 1997, and was accorded ATCC Accession No. 209104. The sequence of BO-55 was determined using an ABI PRISM 377 DNA Sequencer and analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, WI).

<u>Primers Used</u>

BB257: 5'-CTCTCTGCCCTCACCATCCTCGGGAT-3' (Seq. ID No. 21)
BB258: 5'-GACTCCGGCTCGAATACCAGGCAGAG-3' (Seq. ID No. 22)
BB265: 5'-CCATGTTTGCAAAGACCTGGAGGGTCC-3' (Seq. ID No. 23)
BB266: 5'-GGTCACGCGTCAGGAAAGAGACAGCAG-3' (Seq. ID No. 24)
BB172: 5'-AAGCTTCTAGAGATCCCTCGACCTC-3' (Seq. ID No. 25)
BB173: 5'-AGGCGCAGAACTGGTAGGTATGGAA-3' (Seq. ID No. 26)
BB55: 5'-CTTCTAGGCCTGTACGGAAGTGTTA-3' (Seq. ID No. 27)
BB56: 5'-GTTGTGGTTTGTCCAAACTCATCAATG-3' (Seq. ID No. 28)
BB268: 5'-CTGCTGTCTCTTTCCTGACGCGTGACC-3' (Seq. ID No. 29).

<u>Generation of DNA coding for rat $GABA_B$1b and $GABA_B$1a polypeptides</u>

The gene encoding the rat $GABA_B$R1b polypeptide was obtained by screening the same rat hypothalamic library used for $GABA_B$R2 with primers based on the original publication of the clone by Kaupmann, et al., 1997. A partial clone lacking the first 55 nucleotides was identified and ligated to a PCR fragment containing the missing base pairs to obtain the full length clone. A restriction fragment containing the entire coding region of $GABA_B$R1b was subcloned into the mammalian expression vector pEXJ.T7 and designated "BO58". A rat $GABA_B$1a

polypeptide clone was obtained by ligating a restriction fragment of the $GABA_B$1b clone, which contained the common region of the $GABA_B$1 gene, to a PCR product containing the $GABA_B$1a-specific 5' end.

In Situ Hybridization experiments for $GABA_B$R2 mRNA

Animals

Male Sprague-Dawley rats (Charles Rivers, Rochester, NY) were euthanized using CO_2, decapitated, and their brains immediately removed and rapidly frozen on crushed dry ice. Coronal sections of brain tissue were cut at 11 µm using a cryostat and thaw-mounted onto poly-L-lysine-coated slides and stored at -20°C until use.

Tissue Preparation

Prior to hybridization, the tissues were fixed in 4% paraformaldehyde/PBS pH 7.4 followed by two washes in PBS (Specialty Media, Lavallette, NJ). Tissues were then treated in 5 mM dithiothreitol, rinsed in DEPC-treated PBS, acetylated in 0.1 M triethanolamine containing 0.25% acetic anhydride, rinsed twice in 2 x SSC, delipidated with chloroform then dehydrated through a series of graded alcohols. All reagents were purchased from Sigma (St. Louis, MO).

Radioactive *In Situ* Hybridization Histochemistry

Oligonucleotide probes, MJ79/80, corresponding to nucleotides 183-227 and MJ109/110, corresponding to nucleotides 781-820 of the rat $GABA_B$R2 cDNA, MJ94/95, corresponding to nucleotides 151-193 of the human $GABA_B$R1a cDNA, and MJ83/84, corresponding to nucleotides 34-71 of the rat $GABA_B$R1b cDNA were used to characterize the distribution of each polypeptides's respective mRNA. The oligonucleotides were synthesized using an Expedite Nucleic Acid Synthesis System (PerSeptive Biosystems, Framingham, MA) and purified using 12%

polyacrylamide gel electrophoresis. Additionally, sense and antisense oligonucleotides corresponding to positions 1076-1120 of $GABA_BR1b$ (1424-1468 of $GABA_BR1a$) were used (BB403 and BB404).

The sequences of the oligonucleotides are:

For rat $GABA_BR2$:

Sense probe, MJ79:
5'- GCA ATA AAG TAT GGG CTG AAC CAT TTG ATG GTG TTT GGA GGC GT -3' (Seq. ID No. 36)

Antisense probe, MJ80:
5'- ACG CCT CCA AAC ACC ATC AAA TGG TTC AGC CCA TAC TTT ATT GC- 3' (Seq. ID No. 37)

Sense probe, MJ109:
5'- TTT GAG CCC CTG AGC TCC AAA CAA ATC AAG ACC ATC TCA G- 3' (Seq. ID No. 38)

Antisense probe, MJ110:
5'- CTG AGA TGG TCT TGA TTT GTT TGG AGC TCA GGG GCT CAA A- 3' (Seq. ID No. 39)

For human $GABA_BR1a$:

Sense probe, MJ94:
5'- AAG GCC ATC AAC TTC CTG CCT GTG GAC TAT GAG ATC GAA TAT G- 3' (Seq. ID No. 40)

Antisense probe, MJ95:
5'- CAT ATT CGA TCT CAT AGT CCA CAG GCA GGA AGT TGA TGG CCT T- 3' (Seq. ID No. 41)

For rat $GABA_BR1b$:

Sense probe, MJ83:

5'- TGG CCG CTG CCT CTT CTG CTG GTG ATG GCG GCT GGG GT - 3' (Seq. ID No. 42)

Antisense probe, MJ84:
5'- ACC CCA GCC GCC ATC ACC AGC AGA AGA GGC AGC GGC CA -3' (Seq. ID No. 43)

Sense probe, BB403:
5' - CCT TGG CTT TGG CCT TGA ACA AGA CGT CTG GAG GAG GTG GTC GTT -3' (Seq. ID No. 44)

Antisense probe, BB404:
5' - AAC GAC CAC CTC CTC CAG ACG TCT TGT TCA AGG CCA AAG CCA AGG -3' (Seq. ID No. 45)

Probes were 3'-end labeled with [^{35}S]dATP (1200Ci/mmol, NEN, Boston, MA) to a specific activity of 10^9 dpm/μg using terminal deoxynucleotidyl transferase (Pharmacia, Piscataway, NJ). *In situ* hybridization was done with modification of the method described by Durkin, M, et al, 1995.

Nonradioactive *In Situ* Hybridization Histochemistry

Antisense/sense probes corresponding to nucleotides 183 - 227 of the rat $GABA_BR2$ cDNA, were 3'-end labeled with digoxigenin using TdT. The labeling reaction was carried out as outlined in the DIG/Genius System, (Boehringer Mannheim, Indianapolis, IN). Conditions used in ISHH with digoxigenin-labeled probes are the same as described above. The sections were rinsed in buffer 1, washing buffer (0.1 M Tris-HCl pH 7.5/0.15 M NaCl), pre-incubated in Blocking Solution (Buffer 1 , 0.1% Triton-X and 2% normal sheep serum) for 30 minutes and then incubated for

2 hours in Blocking Solution containing anti-digoxigenin-AP Fab fragment (Boehringer Mannheim) at 1:500 dilution followed by two 10 minute washes in Buffer 1. To develop color, sections were rinsed in Detection Buffer (0.1M Tris-HCl pH 9.5/0.15M NaCl/0.05 M $MgCl_2$) for 10 minutes and then incubated overnight in Detection Buffer containing 0.5 mM NBT, 0.1 mM BCIP, and 1 mM levamisole. After color development, slides were dipped in dH_2O and coverslipped using aqua mount.

Probe specificity was established by performing *in situ* hybridization on HEK293 cells transiently transfected with eukaryotic expression vectors containing the rat $GABA_BR1b$ and human $GABA_BR1a$ DNA or no insert for transfection. Furthermore, two pairs of hybridization probes, sense and antisense, that were targeted to different segments of the $GABA_BR2$ mRNA were used for cells and rat tissues.

Quantification

The strength of the hybridization signal obtained in various region of the rat brain was graded as weak (+), moderate (++), heavy (+++) or intense (++++). These were qualitative evaluations for each of the polypeptide mRNA distributions based on the relative optical density on the autoradiographic film and on the relative number of silver grains observed over individual cells at the microscopic level.

Cell Culture

COS-7 cells are grown on 150 mm plates in DMEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 µg/mL streptomycin) at 37°C, 5% CO_2. Stock plates of COS-7 cells are trypsinized and split 1:6 every

3-4 days.

Human embryonic kidney 293 cells are grown on 150 mm plates in DMEM with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100µg/mL streptomycin) at 37°C, 5% CO_2. Stock plates of 293 cells are trypsinized and split 1:6 every 3-4 days.

Mouse fibroblast LM(tk-) cells are grown on 150 mm plates in D-MEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 µg/mL streptomycin) at 37°C, 5% CO_2. Stock plates of LM(tk-) cells are trypsinized and split 1:10 every 3-4 days.

Chinese hamster ovary (CHO) cells are grown on 150 mm plates in HAM's F-12 medium with supplements (10% bovine calf serum, 4 mM L-glutamine and 100 units/mL penicillin/100 ug/mL streptomycin) at 37°C, 5% CO2. Stock plates of CHO cells are trypsinized and split 1:8 every 3-4 days.

Mouse embryonic fibroblast NIH-3T3 cells are grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 µg/mL streptomycin) at 37°C, 5% CO2. Stock plates of NIH-3T3 cells are trypsinized and split 1:15 every 3-4 days.

Sf9 and Sf21 cells are grown in monolayers on 150 mm tissue culture dishes in TMN-FH media supplemented with 10% fetal calf serum, at 27°C, no CO_2. High Five insect cells are grown on 150 mm tissue culture dishes in Ex-Cell 400™ medium supplemented with L-Glutamine, also at 27°C, no CO_2.

LM(tk-) cells stably transfected with the DNA encoding the polypeptides disclosed herein may be routinely converted from an adherent monolayer to a viable suspension. Adherent cells are harvested with trypsin at the point of confluence, resuspended in a minimal volume of complete DMEM for a cell count, and further diluted to a concentration of 10^6 cells/mL in suspension media (10% bovine calf serum, 10% 10X Medium 199 (Gibco), 9 mM $NaHCO_3$, 25 mM glucose, 2 mM L-glutamine, 100 units/mL penicillin/100 µg/mL streptomycin, and 0.05% methyl cellulose). Cell suspensions are maintained in a shaking incubator at 37°C, 5% CO_2 for 24 hours. Membranes harvested from cells grown in this manner may be stored as large, uniform batches in liquid nitrogen.

Alternatively, cells may be returned to adherent cell culture in complete DMEM by distribution into 96-well microtiter plates coated with poly-D-lysine (0.01 mg/mL) followed by incubation at 37°C, 5% CO_2 for 24 hours.

Generation of baculovirus

The coding region of DNA encoding the polypeptides disclosed herein may be subcloned into pBlueBacIII into existing restriction sites, or sites engineered into sequences 5' and 3' to the coding region of the polypeptides. To generate baculovirus, 0.5 µg of viral DNA (BaculoGold) and 3 µg of DNA construct encoding a polypeptide may be co-transfected into 2 x 10^6 *Spodoptera frugiperda* insect Sf9 cells by the calcium phosphate co-precipitation method, as outlined in by Pharmingen (in "Baculovirus Expression Vector System: Procedures and Methods Manual"). The cells then are incubated for 5 days at 27°C.

The supernatant of the co-transfection plate may be

collected by centrifugation and the recombinant virus plaque purified. The procedure to infect cells with virus, to prepare stocks of virus and to titer the virus stocks are as described in Pharmingen's manual.

Transfection

All subtypes studied may be transiently transfected into COS-7 cells by the DEAE-dextran method, using 1 μg of DNA /10^6 cells (Cullen, 1987). In addition, Schneider 2 Drosophila cells may be cotransfected with vectors containing the gene, under control of a promoter which is active in insect cells, and a selectable resistance gene, eg., the G418 resistant neomycin gene, for expression of the polypeptides disclosed herein.

Stable Transfection

DNA encoding the polypeptides disclosed herein may be co-transfected with a G-418 resistant gene into the human embryonic kidney 293 cell line by a calcium phosphate transfection method (Cullen, 1987). Stably transfected cells are selected with G-418.

Radioligand binding assays

Transfected cells from culture flasks were scraped into 5 mL of Tris-HCl, 5mM EDTA, pH 7.5, and lysed by sonication. The cell lysates were centrifuged at 1000 rpm for 5 min. at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 min. at 4°C. The pellet was suspended in binding buffer (50 mM Tris-HCl, 2.5 mM $CaCl_2$ at pH 7.5 supplemented with 0.1% BSA, 2μg/mL aprotinin, 0.5mg/mL leupeptin, and 10μg/mL phosphoramidon). Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10% of the added labeled compound (typically a radiolabeled compound), were added to 96-well

polypropylene microtiter plates containing labeled compound, unlabeled compounds (i.e., displacing ligand in an equilibrium competition binding assay) and binding buffer to a final volume of 250 µL. In equilibrium saturation binding assays membrane preparations were incubated in the presence of increasing concentrations of labeled compound. The binding affinities of the different compounds were determined in equilibrium competition binding assays, using labeled compound, such as 1 nM [^{3}H]-CGP54626, in the presence of ten to twelve different concentrations of the displacing ligand(s). Some examples of displacing ligands included GABA, baclofen, 3APMPA, phaclofen, CGP54626, and CGP55845. Mixtures of several unlabeled test compounds (up to about 10 compounds) may also be used in competition binding assays, to determine whether one of the mixture component compounds binds to the polypeptide or receptor. Binding reaction mixtures were incubated for 1 hr at 30°C, and the reaction was stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Where the labeled compound was a radiolabeled compound, the amount of bound compound was evaluated by gamma counting (for ^{125}I) or scintillation counting (for ^{3}H). Data were analyzed by a computerized non-linear regression program. Non-specific binding was defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of excess unlabeled compound. Protein concentration may be measured by the Bradford method using Bio-Rad Reagent, with bovine serum albumin as a standard.

<u>Cyclic AMP (cAMP) formation assay</u>

The receptor-mediated inhibition of cyclic AMP (cAMP) formation may be assayed in transfected cells expressing the mammalian receptors described herein. Cells are

plated in 96-well plates and incubated in Dulbecco's phosphate buffered saline (PBS) supplemented with 10 mM HEPES, 5mM theophylline, 2 μg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 μg/ml phosphoramidon for 20 min at 37°C, in 5% CO_2. Test compounds are added and incubated for an additional 10 min at 37°C. The medium is then aspirated and the reaction stopped by the addition of 100 mM HCl. The plates are stored at 4°C for 15 min, and the cAMP content in the stopping solution measured by radioimmunoassay. Radioactivity may be quantified using a gamma counter equipped with data reduction software.

Generation of chimeric G-proteins

Chimeric G-proteins were constructed using standard mutagenesis methods (Conklin *et al.*, 1993). Two chimeras were constructed. The first comprises the entire coding region of human $G\alpha_q$ with the exception of the final 3' 15 nucleotides which encode the C-terminal 5 amino acids of $G\alpha_{i3}$. The second also comprises the entire coding region of human $G\alpha_q$ with the exception of the final 3' 15 nucleotides which encode the C-terminal 5 amino acids of $G\alpha_z$. Sequences of both chimeric G-protein genes were verified by nucleotide sequencing. For the purposes of expression in oocytes, synthetic mRNA transcripts of each gene were synthesized using the T7 polymerase.

Phosphoinositide Assay

The agonist activities of GABA-B agonists were assayed by measuring their ability to generate phosphoinositide production in COS-7 cells transfected transiently with $GABA_BR1$, $GABA_BR2$, and chimeric $G\alpha_{q/z}$. Alternatively, COS-7 cells are transfected transiently with $GABA_BR1$, $GABA_BR2$, and other chimeric G-protein alpha subunits such as $G\alpha_{q/i2}$, $G\alpha_{q/i3}$, or $G\alpha_{q/o}$. Cells were plated in 96-well plates and

grown to confluence. The day before the assay the growth medium was changed to 100 µl of medium containing 1% serum and 0.5 µCi [^{3}H]*myo*-inositol, and the plates were incubated overnight in a CO_2 incubator (5% CO_2 at 37°C).

Immediately before the assay, the medium was removed and replaced by 200 µl of PBS containing 10 mM LiCl, and the cells were equilibrated with the new medium for 20 min. The [^{3}H]inositol-phosphate (IP) accumulation was started by adding 22 µl of a solution containing the agonist. To the first two wells 22 µl of PBS were added to measure basal accumulation, and 10 different concentrations of agonist were assayed in the following 10 wells of each plate row. All assays were performed in duplicate by repeating the same additions in two consecutive rows. The plates were incubated in a CO_2 incubator for 30 min. The reaction was terminated by removal of the buffer solution by blotting, followed by the addition of 100 µl of 50% (v/v) trichloroacetic acid (TCA), and 10 min incubation at 4°C.

The contents of the wells were then transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates were prepared adding 100 µl of Dowex AG1-X8 suspension (50% v/v, water:resin) to each well. The filter plates were placed on a vacuum manifold to wash or elute the resin bed. Each well was washed 3 times with 200 µl of 5mM *myo*-inositol. The [^{3}H]-IPs were eluted into empty 96-well plates with 75 µl of 1.2 M ammonium formate/0.1 M formic acid. After the addition of 200 µl of scintillation cocktail (Optiphase Supermix; Wallac) to each well, [^{3}H]-Ips were quantified by counting on a Trilux 1450 Microbeta scintillation counter.

Oocyte expression

Female *Xenopus laevis* (Xenopus-1, Ann Arbor, MI) are anesthetized in 0.2% tricain (3-aminobenzoic acid ethyl ester, Sigma Chemical Corp.) and a portion of ovary is removed using aseptic technique (Quick and Lester, 1994). Oocytes are defolliculated using 3 mg/ml collagenase (Worthington Biochemical Corp., Freehold, NJ) in a solution containing 87.5 mM NaCl, 2 mM KCl, 2 mM $MgCl_2$ and 5 mM HEPES, pH 7.5. Oocytes are injected (Nanoject, Drummond Scientific, Broomall, PA) with 50-70 nl mRNA prepared as described below. After injection of mRNA, oocytes are incubated at 17 degrees for 3-8 days.

RNAs are prepared by transcription from: (1), linearized DNA plasmids containing the complete coding region of the gene, or (2), templates generated by PCR incorporating a T7 promoter and a poly A^+ tail. From either source, DNA is transcribed into mRNA using the T7 polymerase ("Message Machine", Ambion).

The transcription template for the rat $GABA_B$R1b gene was prepared by PCR amplification of the plasmid BO58 using the primers MJ23 and MJ47 (see below). The template for the rat $GABA_B$R2 gene was made by linearization of the plasmid BO56 with NotI.

Primers:

MJ23 5' CCAAGCTTCTAATACGACTCACTATAGGGGAGACCATGGGCCCGGGGGG

ACCCTGTACC 3' (Seq. ID No. 30);

MJ47 5' $T_{(35)}$CACTTGTAAAGCAAATGTACTCGACTCC 3' (Seq. ID No. 31).

Genes encoding G-protein inwardly rectifying K^+ channels 1 and 4 (GIRK1 and GIRK4; "GIRKs") were obtained by PCR using the published sequences (Kubo et al., 1993; Dascal et al., 1993; Krapivinsky et al., 1995b) to derive appropriate 5' and 3' primers. Human heart cDNA was used as template together with the primers

5'-CGCGGATCCATTATGTCTGCACTCCGAAGGAAATTTG-3' (Seq. ID No. 32) and

5'-CGCGAATTCTTATGTGAAGCGATCAGAGTTCATTTTTC -3' (Seq. ID No. 33) for GIRK1 and

5'-GCGGGATCCGCTATGGCTGGTGATTCTAGGAATG-3' (Seq. ID No. 34) and

5'- CCGGAATTCCCCTCACACCGAGCCCCTGG-3' (Seq. ID No. 35) for GIRK4.

The BamH1 and EcoR1 restriction sites in each primer pair were used to clone the PCR product into the expression vector pcDNA-Amp (Invitrogen). Plasmid vectors containing GIRK1 and GIRK4 are referred to as "JS1800" and "JS1741", respectively. The coding regions of both genes were sequenced and verified.

Oocyte electrophysiology

Dual electrode voltage clamp ("GeneClamp", Axon Instruments Inc., Foster City, CA) is performed using 3 M KCl-filled glass microelectrodes having resistances of 1-3 Mohms. Unless otherwise specified, oocytes are voltage clamped at a holding potential of -80 mV. During recordings, oocytes are bathed in continuously flowing (1-3 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.5 (ND96), or elevated K^+ containing 49 mM KCl, 49 mM NaCl, 1.8 mM $CaCl_2$, 2 mM $MgCl_2$, and 5 mM HEPES, pH 7.5 (hK). Drugs are applied either by local perfusion from a 10 µl glass capillary tube fixed at a distance of 0.5 mm from the oocyte, or for calculation of steady-state EC_{50}s, by switching from a series of gravity fed perfusion lines. Experiments are carried out at room temperature. All values are expressed as mean +/- standard error of the mean.

Concentration-response curves for agonists and antagonists were fitted with logistic equations of the form $I = 1/(1 + (EC_{50}/[Agonist])^n)$ for agonists and $I = 1/(1 + ([Antagonist]/IC_{50})^n)$ for antagonists, where I is current, where EC_{50} is the concentration of agonist that produced half-maximal activation, IC_{50} is the concentration of antagonist that produced half-maximal inhibition, and n the Hill coefficient. Fits were made with a Marquardt-Levenberg non-linear least-squares curve fitting algorithm.

Recording ion currents in mammalian cells

The ability of the rat $GABA_BR1$ and $GABA_BR2$ genes to activate GIRK currents in mammalian cells was investigated by transient transfection of HEK-293 cells followed by voltage clamp analysis of currents. HEK-293 cells were maintained in Dulbecco's modified Eagle medium (DMEM) plus 10% (v/v) bovine calf serum, 2% L-glutamine, 50 U/ml penicillin, and 50 µg/ml streptomycin and were incubated at 37° C in a humidified 5% CO_2 atmosphere. Cells were harvested twice each week by treatment with 0.25% trypsin/1 mM EDTA in Hank's Salts and re-seeded at 20% of their original density either into 75 cm^2 flasks (for passaging) or into 35 mm tissue culture dishes (for transfection and electrophysiology experiments).

HEK-293 cells, 40% - 80% confluent, were co-transfected with various combinations of 0.6 ug each of the following plasmids: pGreen Lantern-1 (Gibco/BRL, Gaithersburg, MD), human GIRK1 (JS1800), human GIRK4 (JS1741), rat $GABA_BR1b$ (BO58), and rat $GABA_BR2$ (BO55). Cells were transiently transfected using the Superfect Transfection Reagent from Qiagen (Valencia, CA) according to the manufacturer's instructions. Briefly, 3 µg total plasmid DNA were incubated with 22.5 µl Superfect Reagent in 100 µl serum-free DMEM for 5-10 minutes at room temperature. After addition of 600 µl complete DMEM, the DNA/Superfect mixture was transferred to cells growing in 35 mm dishes coated with poly-D-lysine and incubated for 2-4 hours at 37° C in a 5% CO_2 incubator. Subsequently, the dishes were washed once with phosphate-buffered saline and 2 ml complete DMEM was added. Cells were incubated for 24-72 hours at 37° C before performing electrophysiological measurements.

The whole-cell configuration of the patch-clamp technique was used with glass pipettes having resistances of 2-4 MΩ when filled with the pipette solution. Solutions used were (in mM), $KMeSO_4$, 125; KCl, 5; NaCl, 5; $MgCl_2$, 2; EGTA, 11; HEPES, 10, pH 7.4; MgATP, 1.0; Na_2GTP, 0.2, for the pipette and NaCl, 130; KCl, 4; $CaCl_2$, 2; $MgCl_2$, 2; Glucose, 10; Sucrose, 10; HEPES, 10, pH 7.4 for the bath. GIRK currents were recorded in elevated K^+ solution containing 25 mM K^+ and a correspondingly lower concentration of Na^+. Voltage clamp recordings were made with an EPC-9 amplifier using Pulse+PulseFit software (HEKA Elektronik). Series resistances were kept below 10 Mohm and no attempt was made to provide series resistance compensation. Currents were low-pass filtered at 1 kHz and digitized at a rate of 5 kHz. Unless otherwise noted, experiments were performed at room temperature on cells voltage clamped at a holding potential of -70 mV. Application of agonists was realized using a gravity-fed, perfusion system consisting of six concentrically arranged microcapillary tubes (Jones et al. 1997). The time to complete solution exchange was about 100 ms. The bath was constantly perfused at a low rate with control solution.

All voltage clamp recordings were made from transfected cells visualized under epifluorescent lighting conditions utilizing a filter set designed for GFP (Zeiss Optics). Fluorescent cells were an excellent indication of transfection since they all exhibited some constitutive GIRK current activity in contrast to untransfected cells which displayed no measurable inward rectifier K^+ currents (data not shown).

Microphysiometry

$GABA_BR1$, $GABA_BR2$ or the combination, were transiently expressed in CHO-K1 cells by liposome mediated transfection according to the manufacturer's recommendations ("LipofectAMINE", GibcoBRL, Bethesda, MD), and maintained in Ham's F-12 medium with 10% bovine serum. Cells were prepared for microphysiometric recording as previously described (Salon, J. A., et al., 1995). On the day of the experiment the cell capsules were transferred to the microphysiometer and allowed to equilibrate in recording media (low buffer RPMI 1640, no bicarbonate, no serum, Molecular Devices Corp.), during which a baseline was established. The recording paradigm consisted of a 100 ml/min flow rate and a 30 s flow interruption during which the rate measurement was taken. Challenges involved an 80 s drug exposure just prior to the first post-challenge rate measurement being taken, followed by two additional pump cycles. Acidification rates reported are expressed as a percentage increase of the peak response over the baseline rate observed just prior to challenge.

N-terminal deletion experiments

As a start to exploring the structural aspects of $GABA_BR2$ important for functional activity of the $GABA_BR1/R2$ receptor, N-terminal deletion experiments were performed on the $GABA_BR2$-HA construct (see below). All such deletion mutants caused a complete disruption of receptor activity as assessed by the measurement of GIRK currents in transfected HEK293 cells. In one such experiment, wildtype $GABA_BR2$-HA was digested with BglII restriction enzyme and religated. The BglII deletion mutant (M118) lacks 257 amino acids at the N-terminus, corresponding to positions 169-425. Using immunofluorescence, M118 was

found to be expressed on the cell surface, similarly to the wildtype $GABA_BR2$-HA, yet when co-expressed with $GABA_BR1$ did not produce GIRK activation with 100 µM GABA. Thus, although we cannot yet identify specific amino acids contributing to receptor activity, it appears that the N-terminal region comprising amino acids 169-425 is critically important either for dimer formation, ligand binding or conformational changes associated with signal transduction.

Construction of epitope-tagged polypeptides and confocal microscopy

Incorporation of sequences encoding the RGS6xHis or influenza virus hemagglutinin (HA) epitope into the $GABA_BR1$ and $GABA_BR2$ genes, respectively, was performed by PCR. Each epitope was positioned immediately before the stop codon in the appropriate gene. Both tagged genes were subcloned into pcDNA. Sequence analysis was used to confirm all PCR-derived portions of the construct. Forty-eight hours post-transfection HEK293 cells were fixed for 20 min in 4% paraformaldehyde in PBS, permeablized in PBS containing 2% BSA and 0.1% Triton X-100 and incubated with primary antibody for 1.5 h. Mouse monoclonal anti-RGS (Qiagen) and mouse anti-FLAG (Boehringer-Mannheim) were labeled with FITC-conjugated goat anti-mouse antibodies. Rat monoclonal anti-HA (Boehringer-Mannheim) was visualized with TRITC-conjugated rabbit anti-irat antibodies. Fluorescent images were obtained with a Zeiss LSM 410 confocal microscope using a 100x oil-immersion objective.

Immunoprecipitation and Western blotting

Forty-eight hours following transient transfection HEK293

cells were solubilized in lysis buffer containing (in mM): 50 Tris/Cl pH 7.4, 300 NaCl, 1.5 $MgCl_2$, 1 $CaCl_2$, protease inhibitors (Boehringer Mannheim tablets), 1% Triton X-100, and 10% glycerol. 1-2 mg of protein was immunoprecipitated overnight at 4° C with either 0.5 μg rat monoclonal anti-HA antibody or 0.5 μg mouse monoclonal anti-4xHis antibody (Qiagen). Immune complexes were bound to 20 μl Protein-A agarose (Research Diagnostics, Inc.) for 2 h at RT. Protein-A pellets were washed twice with buffer containing Triton-X-100, then once without, and eluted with 80 μl Laemmli sample buffer containing 2% (w/v) SDS and 20 mM DTT. After heating for 3 min. at 70° C, 20 μl IP samples or 20 μg total protein was subjected to SDS-PAGE followed by Western blotting with either anti-HA or anti-4xHis antibody, followed by sheep anti-rat (Amersham) or goat anti-mouse (RDI) HRP-linked secondary antibodies, respectively. Proteins were visualized with enhanced chemiluminescent substrates (Pierce).

Alternatively, material for immunoprecipitations was obtained by sucrose gradient fractionation of the P1 pellet as described by Graham(Graham, 1984). To verify the enrichment of plasma membrane in the resulting "P1+" pellet, Na^+/K^+ ATPase in the P1+ and P2 (primarily microsomal and vesicular(Graham, 1984)) fractions was quantified by fluorescence detection of anti-alpha 1 subunit antibody (Research Diagnostics, Inc., clone 9A-5) on a phosphor imager (Molecular Dynamics). ATPase in P1+ fractions used for immunoprecipitations was found to be enriched >50 fold compared to P2 fractions.

Experimental Results

Novel GPCR sequences identified by BLAST search

The rat $GABA_BR1a$ amino acid sequence (Kaupmann *et al.* (1997) Nature 386:239) was used as a query to search the EST division of GenBank with BLAST. Two entries, T07621 and Z43654, had probability scores that suggested significant amino acid homology to the $GABA_BR1a$ polypeptide. T07621 had sequence homology from the beginning of the first transmembrane domain to the beginning of third transmembrane domain of the $GABA_BR1a$ polypeptide. Z43654 had sequence homology from the sixth transmembrane domain to the seventh transmembrane domain of the $GABA_BR1a$ polypeptide. The sequence documentation for T07621 and Z43654 was retrieved with Entrez (NCBI) and neither sequence was annotated as having homology to any 7-TM spanning protein.

These results were used to obtain a full-length human clone TL-266, comprising both of the sequences identified by the BLAST search. Sequence analysis of clone TL-266 revealed a complete coding region for a novel protein. A search of the GenEMBL database indicated that the most similar sequence was that of $GABA_BR1a$, followed by G protein-coupled receptors (GPCRs) of the metabotropic receptor superfamily. The nucleotide and deduced amino acid sequence of TL-267 are shown in Figures 1 and 2, respectively. The nucleotide sequence of the coding region is 57% identical to the rat $GABA_BR1a$ over a region of 1,686 bases. The longest open reading frame encodes an 898 amino acid protein with 38% amino acid identity to the rat $GABA_BR1a$ polypeptide. Hydropathy plots of the predicted amino acid sequence reveal seven hydrophobic regions that may represent transmembrane domains (TMs, data not shown), typical of the G protein-coupled receptor superfamily. In the putative TM domains, $GABA_BR2$ exhibits 45% amino acid identity with the rat $GABA_BR1a$

polypeptide. The amino terminus of TL-266 has amino acid homology to the bacterial periplasmic binding protein, a common feature of the metabotropic receptors (O'Hara et al. (1993) Neuron 11:41-52).

Generation of rat $GABA_BR2$ PCR Product

Using PCR primers designed against the first and seventh transmembrane domains of the human $GABA_BR2$ sequence, BB257 and BB258, a 780 base pair fragment was amplified from rat hippocampus and rat cerebellum. Sequence from these bands displayed 90% nucleotide identity to the human $GABA_BR2$ gene. This level of homology is typical of a species homologue relationship in the GPCR superfamily.

Construction and Screening of a Rat Hypothalamic cDNA Library

To obtain a full-length rat $GABA_BR2$ clone, pools of a rat hypothalamic cDNA library were screened by PCR using primers BB265 and BB266. A 440 base pair fragment was amplified from 44 out of 47 pools. Vector-anchored PCR was performed to identify pools with the largest insert size. One positive primary pool, I-47, was subdivided into 24 pools of 1000 individual clones and screened by vector-anchored PCR. Seven positive subpools were identified and one, I-47-4, was subdivided into 10 pools of 200 clones, plated onto agar plates, and screened by southern analysis. Four closely clustering colonies that appeared positive were rescreened individually by vector-anchored PCR. One positive colony, I-47-4-2, designated BO54, was amplified as a single rat $GABA_BR2$ clone. Since vector-anchored PCR revealed that BO54 was in the wrong orientation for expression, the insert was isolated by restriction digest and subcloned into the expression

vector pEXJ. A transformant in the correct orientation was identified by vector-anchored PCR, and designated BO-55.

The nucleotide and deduced amino acid sequence of BO-55 are shown in Figures 3 and 4, respectively. BO-55 contains a 2.6 kB open reading frame and encodes a polypeptide of 883 amino acids. The nucleotide sequence of BO-55 is 89% identical to TL-267 in the coding region, with an overall amino acid identity of 98%.

A BLAST search of GenEMBL indicated that this sequence was most closely related to $GABA_BR1$, displaying 35% and 41% amino acid identities overall and within the predicted transmembrane domains, respectively (Fig. 10). The structural similarity to $GABA_BR1$ indicated that this sequence encodes a novel polypeptide, which we refer to as $GABA_BR2$. The next most related sequences were other members of the mGluR family, with 21-24% overall amino acid identities. Like $GABA_BR1$ and other members of the mGluR family (O'Hara, P. J., et al., 1998), $GABA_BR2$ contains a large N-terminal extracellular domain having regions of homology to bacterial periplasmic binding proteins.

Distribution of $GABA_BR1$ or $GABA_BR2$ in cDNA libraries

Three cDNA libraries were screened by PCR with primers directed to transmembrane regions of either $GABA_BR1$ or $GABA_BR2$. In a human hippocampal cDNA library both polypeptides were found in greater than 90% of the pools and in a rat hypothalamic cDNA library, again both polypeptides were found in greater than 90% of the pools.

In addition, within each of these two libraries, the respective frequency of $GABA_BR1$ and $GABA_BR2$ seems to be the same. However, in a rat spinal cord cDNA library, $GABA_BR1$ was found in 62.5% of the pools while $GABA_BR2$ was found in only 17.5% of the pools. Thus, while both polypeptide subtype appear to be present at high frequency in all three of the libraries, in the spinal cord library $GABA_BR2$ occurs at 3.6-fold lower frequency. These data point to the existence of an additional $GABA_B$-like peptide(s).

<u>Results of Localization</u>

<u>Controls</u>

The specificity of the hybridization of the $GABA_BR2$ probe was verified by performing *in situ* hybridization on transiently transfected HEK293 cells as described in Methods. The results indicate that hybridization to each of the individual $GABA_B$ polypeptides was specific only to the HEK293 cells transfected with each respective cDNA.

In addition, *in situ* hybridization on rat brain sections was performed using two hybridization probes targeted to different segments of the $GABA_BR2$ mRNA. In each case the pattern and intensity of labeling was identical in all regions of the rat CNS. Nonspecific hybridization signal was determined using the sense probes and was indistinguishable from background.

Localization of $GABA_BR2$ mRNA in rat CNS

The anatomical distribution of $GABA_BR2$ mRNA in the rat brain was determined by *in situ* hybridization. By light microscopy the silver grains were determined to be distributed over neuronal profiles. The results suggest that the mRNA for $GABA_BR2$ is widely distributed throughout the rat CNS in addition to several sensory ganglia (Figures 19H-I). However, expression levels in the brain were not uniform with several regions exhibiting higher levels of expression such as the medial habenula, CA3 region of the hippocampus, piriform cortex, and cerebellar Purkinje cells (Figures 19A-F). Moderate expression levels were observed in the ventral pallidum, septum, thalamus, CA1 region of the hippocampus, and geniculate nuclei (Figures 19C,D,E). Lower expression of $GABA_BR2$ mRNA was seen in the hypothalamus, mesencephalon, and several brainstem nuclei (Figures 19D,F). GABAergic neurons and terminals are likewise widely distributed in the CNS (Mugnaini, E., et al., 1985). and the distribution of the $GABA_BR2$ mRNA correlates well with the distribution of GABAergic neurons. One exception is the substantia nigra which contains high densities of GABAergic neurons, yet very low expression of $GABA_BR2$ mRNA. Additionally, the anatomical distribution of $GABA_BR2$ mRNA is in concordance with previous reports of the distribution of $GABA_B$ binding sites obtained using [^{3}H]baclofen (Gehlert, D. R., et al., 1985), and [^{3}H]GABA (Bowery, N. J., et al., 1987). Furthermore, there was a high degree of similarity in the distribution and intensity of $GABA_BR2$ hybridization signal relative to those previously reported for $GABA_BR1$ (Bischoff, S., et al., 1997) (Figures 11, 12). Notable exceptions were the hypothalamus and caudate-putamen, where the expression of $GABA_BR2$ message appeared lower than that of $GABA_BR1$.

Colocalization of $GABA_BR2$and $GABA_BR1b$ mRNAs in the rat CNS

The results of the *in situ* hybridization studies using digoxygenin-labeled probe conjugated to alkaline phosphatase and the chromagen NBT/BCIP for the $GABA_BR2$ mRNA and an [^{35}S]dATP-labeled probe for the $GABA_BR1b$ mRNA indicated that coexpression of the $GABA_BR2$ mRNA and $GABA_BR1b$ mRNA did occur *in vivo* in neurons. In particular, colocalization was observed in cells of the medial habenula, hippocampus, and the cerebellar Purkinje cells. Likewise, there was evidence from the autoradiograms for potential overlapping distribution of the three known $GABA_B$ mRNAs in the olfactory bulb, throughout the entire neocortex, several hypothalamic nuclei, numerous thalamic nuclei and brain stem nuclei. However, the Purkinje cells of the cerebellum contained message for only $GABA_BR2$ and $GABA_BR1b$ and not the $GABA_BR1a$. Additionally, all three subtypes appear to be distributed throughout the gray matter of the spinal cord in all levels of the spinal cord.

The overlapping expression patterns of $GABA_BR1$ and $GABA_BR2$ transcripts in the brain suggested the possibility the polypeptides may be co-expressed in individual neurons and that both might be required for functional activity.

Oocyte expression

Postsynaptic inhibition of neurons by $GABA_B$ receptor activation is caused by the opening of inwardly rectifying K^+ channels (GIRK) (North, R. A., 1989; Andrade, R. et al., 1986; Gahwiler, B. H., et al., 1985; Luscher, C., et al., 1997). Oocytes expressing the combination of $GABA_BR1b$ and $GABA_BR2$ mRNAs together with GIRKs elicited large currents in response to 30 μM GABA

(Table 1a and 1b). (Subsequent to the compilation of the data in Table 1a, experiments were done to make Table 1b.) GABA and baclofen evoked sustained currents of similar magnitude (Fig. 13B). In contrast, oocytes expressing transcripts encoding either $GABA_BR1a$, $GABA_BR1b$, or $GABA_BR2$ alone consistently failed to generate GIRK currents in response to high concentrations of GABA (1 mM), baclofen (1 mM) or 3-APMPA (100 µM). Others have reported similar results with $GABA_BR1$ (Kaupmann, K. et al., 1997a; Kaupmann, K., et al., 1997b).

Table 1a. Magnitude of GIRK currents stimulated by GABA in oocytes and HEK-293 cells expressing GIRK1 and GIRK4 and various combinations of rat $GABA_BR1$ and rat $GABA_BR2$.

	Oocytes mean (nA)	S.E.M.	(n)	**HEK-293** mean (pA)	S.E.M.	(n*)
$GABA_BR1a$	0	0	(35)	-	-	-
$GABA_BR1b$	0	0	(15)	5	3	(3/26)
$GABA_BR2$	0	0	(19)	5	5	(1/6)
$GABA_BR1b$ + $GABA_BR2$	1396	269	(7)	658	323	(9/10)
$GABA_BR1b$ + $GABA_BR2$ + PTX	7	7	(2)	-	-	-

* number of cells responding / total number studied

Table 1b. Magnitude of GIRK currents stimulated by GABA in oocytes and HEK-293 cells expressing GIRK1 and GIRK4 and various combinations of rat GABA$_B$R1 and rat GABA$_B$R2.

	Oocytes			**HEK-293**		
	mean (nA)	S.E.M.	(n)	mean (pA)	S.E.M.	(n*)
GABA$_B$R1a	0	0	(35)	–	–	–
GABA$_B$R1b	0	0	(23)	5	3	(5/26)
GABA$_B$R2	0.230	.13	(30)	.87	.87	(1/23)
GABA$_B$R1b + GABA$_B$R2	832	65	(65)	470	71	(70/81)
GABA$_B$R1b + GABA$_B$R2 + PTX	16	9	(3)	–	–	–

* number of cells responding / total number studied

Currents stimulated by GABA in oocytes injected with both $GABA_BR1b$ and $GABA_BR2$ mRNAs were completely blocked by the selective antagonist CGP55845 (1 µM) in a reversible fashion (data not shown). The potency of GABA and baclofen for eliciting GIRK currents was measured by performing steady-state cumulative concentration response assays on individual oocytes (Figure 6A). Like K^+ responses elicited by stimulation of native $GABA_B$ receptors (Lacy et al. 1988; Misgeld et al. 1995), responses in oocytes did not desensitize and could be faithfully reproduced by multiple agonist applications on single oocytes. Stimulation of inward current was concentration dependent for both GABA and baclofen. The EC_{50}s, 1.76 µM for GABA and 3.99 µM for baclofen (Figure 6B, Figure 7), agreed closely with those reported in the literature for native receptors (Lacy et al. 1988; Misgeld et al. 1995). Concentration-effect curves for GABA were shifted to the right, in an apparently competitive manner, by well characterized $GABA_B$-selective antagonists (Fig. 15B). Based on additional experiments, the EC_{50}'s are 1.32 µM for GABA and 3.31 µM for baclofen. The results to date are summarized in Table 2. Antagonist affinity estimates (Fig. 15B, Table 2) were similar to values reported in previous electrophysiological studies using brain tissue (Bon, C., et al., 1996; Seabrook, G. R., et al., 1990), as well as to those obtained by measuring displacement of radioligand from cells expressing $GABA_BR1$ alone (Kaupmann, K., et al., 1997a) (Table 2).

Table 2. Agonist and antagonist pharmacology in cells expressing $GABA_BR1$, $GABA_BR2$, or both.

Protein	Measurement	Agonist			Antagonist		
		GABA	Baclofen	3-APMPA	Phaclofen	CGP54626	CGP55845
$GABA_BR1$+ $GABA_BR2$	pEC_{50}[1], pK_B[2]	5.88 ±0.01	5.48 ±0.05	7.29 ±0.02	3.80 ± 0.03[4]	7.48 ±0.05	8.60 ±0.09
$GABA_BR1$	pK_i[3]	4.6	4.3	5.2	>3.0	8.95	8.7

[1] n = 6-8 oocytes except for GABA; n = 20 oocytes.

[2] Measured using GABA as agonist; n = 4-6 oocytes.

[3] Displacement of [^{3}H]-CGP54626 from COS-7 cells expressing $GABA_BR1$; n = 3.4

[4] IC_{50} using EC_{50} concentration of GABA.

Evidence that GABA-induced currents were mediated by GIRK channels included: 1) dependency on elevated external K^+, 2) strong inward rectification of the current-voltage (I/V) relation, 3) reversal potential (-23.3 mV) close to the predicted equilibrium potential for K^+ (-23 mV), and 4) sensitivity to block by 100 μM Ba^{++} (Figure 8).

Three oocytes were injected with pertussis toxin (2 ng/oocyte) 6 h before voltage clamping. GABA-stimulated currents were abolished in these oocytes (Table 1a and 1b), suggesting that receptor activation of GIRKs was mediated by G-proteins G_i or G_o. Analogous results have been obtained by others expressing D2 dopamine receptors with GIRKs in oocytes (Werner et al. 1996).

<u>GABA responses in co-transfected HEK-293 cells</u>

To verify that both gene products, $GABA_BR1b$ and $GABA_BR2$, are also required for expression of functional $GABA_B$ receptors in mammalian cells, voltage clamp recordings were obtained from HEK-293 cells transiently transfected with various combinations of each gene along with GIRKs. Cells transfected with a combination of $GABA_BR1b$ (BO58) and $GABA_BR2$ (BO55) plus GIRKs consistently produced large K^+ currents in response to 100 μM GABA (9 of 10 cells tested, Table 1a and 70 of 81 cells tested, Table 1b). Large amplitude currents were also observed when $GABA_BR2$ was paired with the $GABA_BR1a$ splice variant (1046 " 247 pA; n = 9). In contrast, cells transfected with only one of the $GABA_B$ genes plus GIRKs responded either not at all or only very weakly to GABA (Table 1a and 1b). Small agonist-evoked currents (10-50 pA) were observed in 5 of 26 cells expressing $GABA_BR1$; similar weak currents were evoked in 1 of 23 cells expressing $GABA_BR2$.

GABA-elicited currents in doubly transfected cells were completely blocked by 100 μM Ba^{++} or the competitive antagonist CGP55845 at 1 μM (Figure 9). The EC_{50} for GABA stimulation of GIRKs in HEK-293 cells was determined using similar methods as for oocytes. The EC_{50}, 3.42 μM, was comparable to that measured in oocytes (1.76 μM; further experiments gave 1.32 μM). Thus, whether in *Xenopus* oocytes or HEK-293 cells, the behavior of the $GABA_B$ receptor is the same. Co-expression of both $GABA_BR1b$ and $GABA_BR2$ is required to observe activation of the receptor by GABA.

To determine if co-expressed $GABA_BR1/R2$ could mediate a cellular response in the absence of exogenously supplied GIRKs, we transiently co-transfected CHO cells with $GABA_BR1$ and $GABA_BR2$ and measured agonist-evoked extracellular acidification using a microphysiometer. Baclofen stimulated a 9-fold increase in acidification rate (Fig 16) which was blocked by 100 nM CGP55845 and by pretreatment with PTX (not shown). This response was absent in cells expressing either protein alone. Since GIRK activity is undetectable in wild-type CHO cells (Krapivinsky, G., et al., 1995b) we conclude that GIRK expression is not a prerequisite for signal generation by $GABA_BR1/R2$.

$GABA_BR1/GABA_BR2$ signaling through chimeric G-proteins

Chimeric G-proteins have been used to "switch" the coupling pathway of a GPCR from one that normally inhibits adenylyl cyclase to one that activates phospholipase C (Conklin *et al.*, 1993). With the aim of developing an assay based on Ca^{++} or some other signal amenable to high throughput screening, we employed a $G\alpha_{q/i3}$ chimera to obtain Ca^{++}-induced Cl^- responses in oocytes. Oocytes were injected with $GABA_BR1$ and $GABA_BR2$ mRNAs as previously described. 2-3 days later oocytes were

injected again with 50 pg of $G\alpha_{q/i3}$ mRNA and recorded under voltage clamp conditions. In response to GABA (0.1 - 1 mM) 88% of these oocytes produced rapidly desensitizing inward currents (454 ± 92 nA; n = 14) typical of those stimulated by receptors that normally couple to $G\alpha_q$. In contrast, oocytes injected with only the $GABA_BR1/GABA_BR2$ combination (n > 100), or $GABA_BR1$ plus $G\alpha_{q/i3}$ (n = 4) failed to produce currents.

$GABA_B$ agonists also resulted in concentration-dependent stimulation of phosphoinositide production in COS-7 cells transfected transiently with $GABA_BR1$, $GABA_BR2$, and the chimeric G-protein $G\alpha_{q/z}$. The concentration of agonist evoking 50% of its maximum response (EC_{50}) and fold stimulation over basal were: GABA (EC_{50} = 1.8 μM; 2.4 fold); baclofen (1.7 μM; 1.8 fold); 3-aminopropylmethylphosphinic acid (EC_{50} = 0.11 μM; 2.2 fold). These results indicate that G-protein chimeras, in particular $G\alpha_{q/z}$ and $G\alpha_{q/i3}$, are useful for directing $GABA_B$ receptor stimulation to a phosphoinositide- or Ca^{++}-based assay.

A comparison of the pharmacological properties of $GABA_BR1$ and $GABA_BR2$ using radioligand binding revealed that membranes from HEK293 or COS-7 cells expressing $GABA_BR1$, but not those expressing $GABA_BR2$, were labeled by the high affinity antagonist [^{3}H]-CGP54626[21] (Table 2), indicating that the polypeptides are pharmacologically distinct. Neither was labeled by the agonists [^{3}H]-GABA or [^{3}H]-baclofen. Furthermore, with the available ligands (GABA, baclofen, APMPA, phaclofen, CGP54626, CGP-55845 and SCH-50911) the binding profile of membranes from cells co-transfected with $GABA_BR1/R2$ was not different from those transfected with $GABA_BR1$ alone. The absence of detectable high affinity agonist binding to $GABA_BR1/R2$, as well as to

$GABA_BR1b$, constitutes a notable distinction from the $GABA_B$ binding profile in the CNS and may reflect the absence of an essential, as yet undefined G-protein or accessory protein.

The molecular mechanism by which protein co-expression confers functional activity is unknown. We noted that varying the ratios of $GABA_BR1/R2$ cDNAs from 1/100 to 100/1 in HEK293 cells resulted in a symmetrical fall off in response amplitude (Fig.14B). This suggests that a 1:1 protein stoichiometry may be critical, and caused us to postulate that the polypeptides are forming a heteromeric association. Biochemical evidence supports the idea that certain GPCRs can exist as homodimers (Hebert, T.E., et al., 1996; Cvejic, S., et al., 1997; Ciruela, F., et al., 1995; Avissar, S., et al., 1983; Romano, C., et al., 1996), but the functional significance of this has been largely unexplored (Hebert, T.E., et al., 1996; Wreggett, K.A., et al., 1995). The possibility of a physical association was investigated using epitope-tagged versions of $GABA_BR1$ (RGS6xH tag) and $GABA_BR2$ (HA tag). C-terminal modification did not appear to alter the function of either polypeptide; maximal current amplitudes (Fig. 14B) and EC_{50} values for GABA (4.97 μM, n = 5) were unchanged compared to HEK293 cells expressing the wild-type $GABA_BR1/R2$ receptor combination (3.42 μM, n = 5). The subcellular distribution of epitope-tagged proteins was examined in transfected cells by fluorescence microscopy. When expressed individually, $GABA_BR1^{RGS6xH}$ and $GABA_BR2^{HA}$ were localized throughout the plasma membrane. Optical sectioning of antibody-labeled cells by confocal microscopy confirmed the membrane localization pattern, with less labeling in the cytoplasm and none in the nucleus. In co-transfected cells there was a striking overlap in the distribution of the two epitope tags (Fig. 17A-17C). Both proteins were

prominently expressed on the plasma membrane. Furthermore, co-localization occurred within the cytoplasm, suggesting that $GABA_BR1$ and $GABA_BR2$ assemble in the endoplasmic reticulum. In contrast, the cellular distribution of an unrelated GPCR, NPY Y5, differed considerably from that of $GABA_BR2$ (Fig. 17D), suggesting specificity in the association of $GABA_BR2$ with $GABA_BR1$.

Western blots of whole cell extracts from cells expressing $GABA_BR1^{RGS6xH}$, $GABA_BR2^{HA}$ or both, exhibited bands close to the predicted molecular weights of the two proteins (92 kD for $GABA_BR1$, 97 kD for $GABA_BR2$) and additional bands corresponding to the predicted molecular weights of receptor dimers (Fig. 18A,B). To determine if $GABA_BR1$ and $GABA_BR2$ co-associate in a heteromeric complex, we immunoprecipitated solubilized material from cells expressing both polypeptides. $GABA_BR2^{HA}$ was detected in material immunoprecipitated using either anti-His or anti-HA antibodies (Fig. 18). To determine if $GABA_BR1b$ and $GABA_BR2$ co-associate in a heteromeric complex, we performed immunoprecipitations using membrane fractions enriched in plasma membrane as determined by the presence of Na^+/K^+ ATPase (Figure 20A). In co-transfected cells only, $GABA_BR2^{HA}$ was detected in material immunoprecipitated using antibodies specific for the $GABA_BR1^{RGS6xH}$ protein (Figure 20B). This result confirms that both $GABA_BR1$ and $GABA_BR2$ are correctly targeted to the plasma membrane of HEK293 cells, and that the two proteins exist in a heteromeric complex, perhaps as heterodimers, on the membrane surface.

Experimental Discussion

A gene has been cloned that shows 38% overall identity at the amino acid level with the recently cloned $GABA_BR1$ polypeptide. Important predicted features of the new gene product include 7 transmembrane spanning regions, and a large extracellular N-terminal domain. Like the $GABA_BR1$ gene product, $GABA_BR2$ by itself does not promote the activation of cellular effectors such as GIRKs. When co-expressed together, however, the two permit a $GABA_B$ receptor phenotype that is quite similar to that found in the brain. The functional attributes of this reconstituted receptor include: 1) robust stimulation of a physiological effector (GIRKs), 2) EC_{50}s for GABA and baclofen in the same range as for $GABA_B$ receptors previously studied in the CNS, 3) antagonism by the high affinity selective antagonist CGP55845, and 4) inhibition of receptor function by pertussis toxin. These attributes are not observed when either $GABA_BR1$ or $GABA_BR2$ is expressed alone.

Our data indicate that $GABA_BR1$ and $GABA_BR2$ associate as subunits to produce a single pharmacologically and functionally defined receptor. Consistent with this view, double labeling *in situ* hybridization experiments provided evidence that $GABA_BR1$ and $GABA_BR2$ mRNAs are co-expressed in individual neurons and populations of neurons in several regions of the nervous system including hippocampal pyramidal cells (Fig. 21), cerebellar Purkinje cells (Fig. 12A,B) and sensory neurons in mesencephalic trigeminal nucleus (Fig. 21) and dorsal root ganglia. This co-localization pattern of $GABA_BR1$ and R2 transcripts predicts that $GABA_B$ receptors on these cells are comprised of $GABA_BR1/R2$ heteromers. Other as yet unidentified $GABA_B$ receptor homologues may associate elsewhere to produce novel subtypes. For example, the low level of expression of $GABA_BR2$ mRNA relative to $GABA_BR1$ in caudate putamen and hypothalamus (Fig. 11A,B) raises the possibility that other $GABA_B$ receptor homologues may associate with $GABA_BR1$ to produce novel subtypes in these regions. Conclusive evidence that functional $GABA_B$ receptors exist in vivo as multimers will await immunofluorescence studies with specific antibodies.

The recent cloning of a family of accessory proteins that modify the binding and functional properties of a calcitonin-receptor-like receptor (McLarchie, et al., 1998) demonstrates that some 7-TM spanning proteins require additional unrelated proteins to reconstitute native GPCR activity. $GABA_BR1$ and $GABA_BR2$ are the first examples of 7-TM proteins for which activity is dependent on an interaction with another member within the same family of proteins. There will be considerable interest in whether other GPCRs are formed by heteromeric

complexes of related 7-TM proteins. Many members of the superfamily of GPCRs, such as D_3, 5-HT_5, and olfactory receptors, do not function well in heterologous expression systems and may require related partners to generate native receptor function (Nimschinsky, et al., 1997). The growing list of receptors that have been reported to exist as homodimers (Ciruela, F., et al., 1995; Cvejic, S., et al., 1997; Hebert, T.E., et al, 1996; Romano, C., et al., 1996; Maggio, R., et al., 1996) points to the likelihood that both homomeric and heteromeric assemblies are more widespread among GPCRs than previously thought.

There are several possible explanations for why two genes are required for full function of the $GABA_B$ receptor. One possible explanation is that the two gene products function together as a heterodimer having high affinity agonist and antagonist binding sites. Currently, there is no precedent for heterodimerization of GPCRs. There is evidence that certain GPCRs, for example the mGluR5 receptor, can form homodimers via cystine disulfide bridges in the N-terminal domain (Romano et al., 1996). Significantly, synthetic peptides that inhibit homodimerization of beta2-adrenergic receptors also reduce agonist stimulation of adenylyl cyclase activity (Hebert et al., 1996). Useful parallels may be drawn from other classes of receptors where heterodimeric structures are well-known. For example, the NMDA (glutamate) receptor is comprised of two principal subunits, neither of which alone permits all of the native features of the receptor (see Wisden and Seeburg, 1993). $GABA_B$ receptors may be comprised similarly of two (or more) peptide subunits, such as $GABA_BR1$ and $GABA_BR2$, that form a quaternary structure having appropriate

binding sites for agonist and G-protein.

A role for $GABA_BR2$ in modulating sensory information is suggested by *in situ* hybridization histochemistry which revealed the expression of $GABA_BR2$ mRNA in relay nuclei of several sensory pathways. In the olfactory and visual pathways $GABA_BR2$ appears to be in a position to modulate excitatory glutamatergic projections from the olfactory bulb and retina $GABA_BR2$ mRNA was observed in the target regions of projection fibers from the main olfactory bulb, including the olfactory tubercle, piriform and entorhinal cortices and from the retina, for instance the superior colliculus (Figures 19A,B; Table 3).

The ability to modulate nociceptive information might be indicated not only by the presence of $GABA_BR2$ transcripts in somatic sensory neurons of the trigeminal and dorsal root ganglia (Figures 19H-I) but also by being present in the target regions of nociceptive primary afferent fibers, including the superficial layers of the spinal trigeminal nucleus and dorsal horn of the spinal cord (Figures 19F-G). Again, in each of these loci $GABA_BR2$ has been shown to be in a position to potentially modulate the influence of excitatory glutamatergic nociceptive primary afferents. In both ganglia, microscopic examination indicated that the hybridization signal did not appear to be restricted to any one size cell and was distributed evenly over small, medium and large ganglion cells. Thus, $GABA_BR2$ may be able to influence various sensory modalities. Expression levels appeared to be higher in the ganglion cells of the dorsal root with light to moderate expression in the trigeminal ganglia.

$GABA_BR2$ mRNA was likewise observed to be expressed in the vestibular nuclei which are target regions of inhibitory GABAergic Purkinje cells and also in the Purkinje cells themselves, suggesting that $GABA_BR2$ may be important in the mediation of planned movements (Figure 19F).

Moderate expression of $GABA_BR2$ transcripts throughout the telencephalon indicate a potential modulatory role in the processing of somatosensory and limbic system (entorhinal cortex) information, in addition to modulating visual (parietal cortex) and auditory stimuli (temporal cortex) as well as cognition. Furthermore, modulation of patterns of integrated behaviors, such as defense, ingestion, aggression, reproduction and learning could also be attributed to this receptor owing to its expression in the amygdala (Table 3). The high levels of expression in the thalamus suggest a possible regulatory role in the transmission of somatosensory (nociceptive) information to the cortex and the exchange of information between the forebrain and midbrain limbic system (habenula). The presence of $GABA_BR2$ mRNA in the hypothalamus indicates a likely modulatory role in food intake, reproduction, the expression of emotion and possibly neuroendocrine regulation (Figure 19D). A role in the mediation of memory acquisition and learning may be suggested by the presence of the $GABA_BR2$ transcript throughout all regions of the hippocampus and the entorhinal cortex (Figure 19D).

Table 3. Distribution of $rGABA_BR2$, $rGABA_BR1a$, and $GABA_B1b$ mRNA in the rat CNS. The strength of the hybridization signal for each of the respective mRNAs obtained in various regions of the rat brain was graded as weak (+), moderate (++),heavy (+++) or intense (++++)and is relative to the individual polypeptides.

Region	$GABA_BR2$	$GABA_BR1a$*	$GABA_BR1b$*	Potential Application
Olfactory bulb				Modulation of olfactory sensation
internal granule layer	+	++	++	
glomerular layer	+	++	++	
external plexiform layer	-	-	-	
mitral cell layer	-	+	++	
anterior olfactory n	++	++	++	
olfactory tubercle	+	++	+++	
Islands of Calleja	-	++	+++	
Telencephalon				Sensory integration
taenia tecta	++	++	++	
frontal cortex	++	++	++	
orbital cortex	++	++	++	
agranular insular cortex	+++	++	++	

cingulate cortex	++	++	+	
Region	**$GABA_BR2$**	**$GABA_BR1a$***	**$GABA_BR1b$***	**Potential Application**
retrosplenial cortex	++	++	+	
parietal cortex	++	++	++	Processing of visual stimuli
occipital cortex	++	++	++	
temporal cortex	++	++	++	Processing of auditory stimuli
perirhinal cortex	++	++		
entorhinal cortex	++	++	++	Processing of visceral information
dorsal endopiriforn n	++	++	++	
piriform cortex	+++	+++	+++	Integration/ transmission of incoming olfactory information
Basal Ganglia				
accumbens n	+	++	++	Modulation of dopaminergic function
caudate-putamen	+	+	++	Sensory/motor integration
globus pallidus	+	-	+	
Septum				

medial septum	++	++	+	Cognitive enhancement via cholinergic system
Region	**GABA$_B$R2**	**GABA$_B$R1a***	**GABA$_B$R1b***	**Potential Application**
lateral septum	++	+	++	Modulation of integration of stimuli associated with adaptation
septohippo-campal n	+	+	+++	
diagonal band n	++	++	++	
ventral pallidum	++	+	+	
Amygdala				Anxiolytic (activation - reduction in panic attacks) appetite, depression
basolateral n	++	+	+	
medial amygdaloid n	+	+	+	Olfactory amygdala
basomedial n	+	+		
central n	+++	-	+	
anterior cortical n	+	+	+	
postero-medial cortical n	++	+	+	
bed n stria terminalis	++	+	++	

zona incerta	+	+	+	
Hippocampus				Memory consolidation and retention
CA1,Ammon's horn	++	+++	+++	
CA2,Ammon's horn	++++	+++	+++	
Region	**$GABA_BR2$**	**$GABA_BR1a$***	**$GABA_BR1b$***	**Potential Application**
CA3,Ammon's horn	++++	+++	+++	Facilitation of LTP
subiculum	+	+++	+++	
parasubiculum	++	++	++	
presubiculum	++	++	++	
dentate gyrus	++++	+++	++	
polymorph dentate gyrus	+++	+++	++	
Hypothalamus				
suprachiasmatic n	+	++	ND	
median preoptic area	+	+	++	Regulation of gonadotropin secretion and reproductive behaviors
paraventricular n	+	++	++	Appetite/obesity
arcuate n	++	++	++	
anterior hypoth, post	+	+		
lateral hypoth	+	+	++	
ventromedial n	+	++	+++	
periventricular n	+	+	+	
supraoptic n	+	++	+	Synthesis of OXY and AVP

supramammillary n	++	++	++	Modulation of hypothalamic projections to cortex
premammillary n	+	+	+	
medial mammillary n	+	++	+	
Region	**$GABA_BR2$**	**$GABA_BR1a$***	**$GABA_BR1b$***	**Potential Application**
Thalamus				Analgesia/Mod-ulation of sensory information
paraventricular n	++	+	++	Modulation of motor and behavioral responses to pain
centromedial n	++	+	++	Modulation of motor and behavioral responses to pain
paracentral n.	++	+	++	
parafascicular n	++	+	++	Modulation of motor and behavioral responses to pain
anterodorsal n	+++	+	++	Modulation of eye movement
laterodorsal n	+++	+	++	
lateral posterior n	++	+	++	

reuniens n	+++	+	++	Modulation of thalamic input to ventral hippocampus and entorhinal ctx
rhomboid n	+++	+	++	
medial habenula	++++	+	++++	Anxiety/sleep disorders/ analgesia in chronic pain
lateral habenula	+	+	+++	
Region	**GABA$_B$R2**	**GABA$_B$R1a***	**GABA$_B$R1b***	**Potential Application**
ventrolateral n	+++	+	++	
ventromedial n	+++	++	++	
ventral posterolateral n	+++	+	++	
reticular n	++	+	+	Alertness /sedation
lateral geniculate n	++	+	++	Modulation of visual perception
medial geniculate	++	+	++	Modulation of auditory system
subthalamic n	++	++	++	
Mesencephalon				
superior colliculus	+	+	+	Modulation of vision
inferior colliculus	+	+	+	
central gray	+	+	+	Analgesia
dorsal raphe	+	++	+	

deep mesence-phalic n	+	+	+	
oculomotor n	+			
pontine n	+++		++	
retrorubral field	+			
ventral tegmental area	+	++	++	Modulation of the integration of motor behavior and adaptive responses

Region	$GABA_BR2$	$GABA_BR1a$*	$GABA_BR1b$*	Potential Application
substantia nigra, reticular	+	+	+	Motor control
substantia nigra, compact	++	++	++	
interpeduncular n	++	ND	ND	Analgesia
Myelence-phalon				Analgesia
raphe magnus	++		++	
raphe pallidus	+	++	ND	
principal trigeminal	+	+		
spinal trigeminal n	+	+	+	
pontine reticular n	++	+	++	
parvicell-ular reticular n	+	++	++	

locus coeruleus	++	++	++	Modulation of NA transmission
parabrachial n	+	++	+	Modulation of visceral sensory information
vestibular n	+	++	+	Maintenance of balance and equilibrium
gigantocellular reticular n	+	++	++	Inhibition and disinhibition of brainstem
Region	**$GABA_BR2$**	**$GABA_BR1a$***	**$GABA_BR1b$***	**Potential Application**
prepositus hypoglossal n	+	+++	++	Position and movement of the eyes/ Modulation of arterial pressure and heart rate
ventral cochlear n	++	+	ND	
n soltary tract	++			Hypertension
A5 Nor-adrenaline cells	+	ND	ND	
facial n(7)	+	++	+	
Cerebellum				Motor coordination, Autism
granule cell layer	+	+	+	
Purkinje cells	++	-	++	

Spinal cord				Analgesia
dorsal horn	+	++	+	
ventral horn	+	++	+	
trigeminal ganglion	++	+++	+	Nociception
dorsal root ganglion	++++	+++	ND	Nociception

ND = not determined

*Bischoff S *et al.*

List of Abbreviations

7	facial n
ac	anterior commisure
Acb	accumbens n
ACo	anterior cortical amygdaloid n
AI	agranular insular cortex
AON	anterior olfactory n
APir	amygdalopiriform transition area
APT	anterior pretectal n
Arc	arcuate hypothalamic n
BLA	basolateral amygdaloid n
CA1-3	Fields of Ammon's horn
cc	corpus callosum
Cg	cingulate cortex
CeA	central amygdaloid n
CPu	caudate-putamen
DG	dentate gyrus
DLG	dorsal lateral geniculate n
DpMe	deep mesencephalic n
Ent	entorhinal cortex
Gi	gigantocellular reticular n
Gr	granule cll layer, cerebellum
GrO	granule layer olf. bulb
FrA	frontal association cortex
GP	globus pallidus
HDB	horizontal diagonal band
LA	lateral amygdaloid n
LH	lateral hypothalamus
LO	lateral orbital cortex
LV	lateral ventricle
M1	primary motor cortex

MeAD	medial amygdaloid n, anterodorsal
MG	medial geniculate
MHb	medial habenular n
MPO	medial preoptic n
PC	Purkinje cell layer of the cerebellum
PF	parafascicular n
Pir	piriform cortex
PMCo	posteromedial cortical amygdaloid n
Pr	prepositus n
PVA	paraventricular thalamic n
RS	retrosplenial cortex
S	subiculum
SFi	septofimbrial n
SI	substantia innominata
SNc	substantia nigra, compact
STh	subthalamic n
Sp5	spinal trigeminal n
TT	tenia tecta
Ve	vestibular n
VTA	ventral tegmental area

<u>Potential therapeutic application for $GABA_B$ agonists and antagonists</u>

<u>Agonists</u>

Antinociception

A potential $GABA_B$ agonist application may in antinociception. The inhibitory effects of GABA and $GABA_B$ agonists are thought to be predominantly a presynaptic mechanism on excitation-induced impulses in high

threshold Aδ and C fibers on primary afferents. This effect can be blocked by $GABA_B$ antagonists (Hao, J-H., et al., 1994). Baclofen's spinal cord analgesic effects have been well documented in the rat, though it has not been as effective in human. However, baclofen has been successful in the treatment of trigeminal neuralgia in human.

The localization of the $GABA_BR2$ mRNA in the superficial layers of the spinal cord dorsal horn, the termination site for primary afferents, as well as their cells of origin in the dorsal root and trigeminal ganglia position the $GABA_BR1/R2$ receptor appropriately for mediating the agonist effects.

Drug Addiction

It has been suggested that GABA agonists may have some potential in the treatment of cocaine addiction. A role for the action of psychostimulants in the mesoaccumbens dopamine system is well established. The ventral pallidum receives a GABAergic projection from the nucleus accumbens and both regions contain $GABA_BR2$ transcripts. GABA receptors were shown to have an inhibitory effect on dopamine release in the ventral pallidum. Phaclofen acting at these receptors resulted in increased dopamine release and baclofen was shown to attenuate the reinforcing effects of cocaine. (Roberts, D. C. S., et al.,1996; Morgan,A.E. et al.)

Micturition

There is a potential application for $GABA_B$ agonists in the treatment of bladder dysfunction. Baclofen has been used in the treatment of detrussor hyperreflexia through inhibition of contractile responses. In addition to a peripheral site of action for $GABA_B$ agonists, there is also the possibility for a central site. The pontine micturition center in the brainstem is involved in mediating the spinal reflex pathway, via Onuf's nucleus in the sacral spinal cord. Support for possible application of $GABA_B$ agonists in the treatment of bladder dysfunction may be augmented by presence of $GABA_BR2$ mRNA in the various nuclei involved in the control of the lower urinary tract function.

Antagonists

Memory Enhancement - Alzheimer's Disease

$GABA_B$ antagonists may have a potential application in the treatment of Alzheimer's Disease. The blockade of $GABA_B$ receptors might lead to signal amplification and improvement in cognitive functions resulting from an increased excitability of cortical neurons via amplification of the acetycholine signal. Additionally, memory may be enhanced by $GABA_B$ antagonists which have been shown to suppress late IPSPs, thus facilitating long-term potentiation in the hippocampus (see Table 3).

To support this idea, CGP36742, a $GABA_B$ antagonist, has been shown to improve learning performance in aged rats as well as the performance of rhesus monkeys in conditioned spatial color task. (Mondadori, C. et al., 1993). The significance of the $GABA_BR1/R2$ receptor in

cognitive functioning might be indicated by the presence of $GABA_BR2$ mRNA in the cerebral cortex and its codistribution in the ventral forebrain with cortically projecting cholinergic neurons as well as its localization in the pyramidal cells in all regions of Ammon's horn and dentate gyrus in the hippocampus.

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What is claimed is:

1. An isolated nucleic acid encoding a $GABA_BR2$ polypeptide.

2. The nucleic acid of claim 1, wherein the nucleic acid is DNA.

3. The DNA of claim 2, wherein the DNA is cDNA.

4. The DNA of claim 2, wherein the DNA is genomic DNA.

5. The nucleic acid of claim 1, wherein the nucleic acid is RNA.

6. The nucleic acid of claim 1, wherein the nucleic acid encodes a mammalian $GABA_BR2$ polypeptide.

7. The nucleic acid of claim 1, wherein the nucleic acid encodes a rat $GABA_BR2$ polypeptide.

8. The nucleic acid of claim 1, wherein the nucleic acid encodes a human $GABA_BR2$ polypeptide.

9. The nucleic acid of claim 6, wherein the nucleic

acid encodes a polypeptide characterized by an amino acid sequence in the transmembrane regions which has an identity of 90% or higher to the amino acid sequence in the transmembrane regions of the human $GABA_BR2$ polypeptide shown in Figures 5A-5D.

10. The nucleic acid of claim 6, wherein the nucleic acid encodes a mammalian $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as does the $GABA_BR2$ polypeptide encoded by the plasmid BO-55 (ATCC Accession No. 209104).

11. The nucleic acid of claim 7, wherein the nucleic acid encodes a rat $GABA_BR2$ polypeptide which has an amino acid sequence encoded by the plasmid BO-55 (ATCC Accession No. 209104).

12. The nucleic acid of claim 7, wherein the nucleic acid encodes a rat $GABA_BR2$ polypeptide having substantially the same amino acid sequence as the amino acid sequence shown in Figures 4A-4D (Seq. ID No. 4).

13. The nucleic acid of claim 7, wherein the nucleic acid encodes a rat $GABA_BR2$ polypeptide having the amino acid sequence shown in Figures 4A-4D (Seq. ID No. 4).

14. The nucleic acid of claim 6, wherein the nucleic acid encodes a mammalian $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as

does the $GABA_BR2$ polypeptide encoded by the plasmid TL-267 (ATCC Accession No. 209103).

15. The nucleic acid of claim 8, wherein the nucleic acid encodes a human $GABA_BR2$ polypeptide which has an amino acid sequence encoded by the plasmid TL-267(ATCC Accession No. 209103).

16. The nucleic acid of claim 8, wherein the human $GABA_BR2$ polypeptide has a sequence, which sequence comprises substantially the same amino acid sequence as the sequence shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

17. The nucleic acid of claim 8, wherein the human $GABA_BR2$ polypeptide has a sequence, which sequence comprises the sequence shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

18. A purified $GABA_BR2$ protein.

19. A vector comprising the nucleic acid of claim 1.

20. A vector comprising the nucleic acid of claim 8.

21. A vector of claim 19 adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the nucleic

acid in the bacterial cell operatively linked to the nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

22. A vector of claim 19 adapted for expression in an amphibian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the amphibian cell operatively linked to the nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

23. A vector of claim 19 adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the nucleic acid in the yeast cell operatively linked to the nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

24. A vector of claim 19 adapted for expression in an insect cell which comprises the regulatory elements necessary for expression of the nucleic acid in the insect cell operatively linked to the nucleic acid encoding the $GABA_BR2$ polypeptide so as to permit expression thereof.

25. A vector of claim 24 which is a baculovirus.

26. A vector of claim 19 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the

nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

27. A vector of claim 19 wherein the vector is a plasmid.

28. The plasmid of claim 27 designated BO-55 (ATCC Accession No. 209104).

29. The plasmid of claim 27 designated TL-267(ATCC Accession No. 209103).

30. A method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within one of the two strands of the nucleic acid encoding the $GABA_BR2$ polypeptide contained in plasmid BO-55, and detecting hybridization of the probe to the nucleic acid.

31. A method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within (a) the

nucleic acid sequence shown in Figures 1A-1E (Seq. ID No. 1) or (b) the reverse complement to the nucleic acid sequence shown in Figures 1A-1E (Seq. ID No. 1), and detecting hybridization of the probe to the nucleic acid.

32. A method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within one of the two strands of the nucleic acid encoding the $GABA_BR2$ polypeptide contained in plasmid TL-267, and detecting hybridization of the probe to the nucleic acid.

33. A method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within (a) the nucleic acid sequence shown in Figures 3A-3D (Seq. ID No. 3) or (b) the reverse complement to the nucleic acid sequence shown in Figures 3A-3D (Seq. ID No. 3), and detecting hybridization of the probe to the nucleic acid.

34. The method of any one of claims 30 to 33, wherein the nucleic acid is DNA.

35. The method of any one of claims 30 to 33, wherein the nucleic acid is RNA.

36. The method of any one of claims 30 to 33, wherein the probe comprises at least 15 nucleotides complementary to a unique segment of the sequence of the nucleic acid molecule encoding the $GABA_BR2$ polypeptide.

37. A method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising a nucleic acid of at least 15 nucleotides which is complementary to the antisense sequence of a unique segment of the sequence of the nucleic acid encoding the $GABA_BR2$ polypeptide, and detecting hybridization of the probe to the nucleic acid.

38. A method of inhibiting translation of mRNA encoding a $GABA_BR2$ polypeptide which comprises contacting such mRNA with an antisense oligonucleotide having a sequence capable of specifically hybridizing to the mRNA of claim 5, so as to prevent translation of the mRNA.

39. A method of inhibiting translation of mRNA encoding a $GABA_BR2$ polypeptide which comprises contacting such mRNA with an antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA of claim 4.

40. The method of claim 38 or 39, wherein the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.

41. An isolated antibody capable of binding to a $GABA_BR2$ polypeptide encoded by the nucleic acid of claim 1.

42. The antibody of claim 41, wherein the $GABA_BR2$ polypeptide is a human $GABA_BR2$ polypeptide.

43. An antibody capable of competitively inhibiting the binding of the antibody of claim 41 to a $GABA_BR2$ polypeptide.

44. An antibody of claim 41, wherein the antibody is a monoclonal antibody.

45. A monoclonal antibody of claim 44 directed to an epitope of a $GABA_BR2$ polypeptide present on the surface of a $GABA_BR2$ polypeptide expressing cell.

46. A method of claim 38 or 39, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

47. A method of claim 46, wherein the substance which inactivates mRNA is a ribozyme.

48. A pharmaceutical composition which comprises an amount of the antibody of claim 41 effective to block binding of a ligand to the $GABA_BR2$ polypeptide and a pharmaceutically acceptable carrier.

49. A transgenic, nonhuman mammal expressing DNA encoding a $GABA_BR2$ polypeptide of claim 1.

50. A transgenic, nonhuman mammal comprising a homologous recombination knockout of the native $GABA_BR2$ polypeptide.

51. A transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a $GABA_BR2$ polypeptide of claim 1 so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding such $GABA_BR2$ polypeptide and which hybridizes to such mRNA encoding such $GABA_BR2$ polypeptide, thereby reducing its translation.

52. The transgenic, nonhuman mammal of claim 49 or 50, wherein the DNA encoding the $GABA_BR2$ polypeptide additionally comprises an inducible promoter.

53. The transgenic, nonhuman mammal of claim 49 or 50, wherein the DNA encoding the $GABA_BR2$ polypeptide additionally comprises tissue specific regulatory elements.

54. A transgenic, nonhuman mammal of any one of claims 49, 50 or 51, wherein the transgenic, nonhuman mammal is a mouse.

55. A method of detecting the presence of a $GABA_BR2$ polypeptide on the surface of a cell which comprises contacting the cell with the antibody of claim 41 under conditions permitting binding of the antibody to the polypeptide, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a $GABA_BR2$ polypeptide on the surface of the cell.

56. A method of preparing the purified $GABA_BR2$ polypeptide of claim 18 which comprises:

a. inducing cells to express a $GABA_BR2$ polypeptide;

b. recovering the polypeptide so expressed from the induced cells; and

c. purifying the polypeptide so recovered.

57. A method of preparing the purified $GABA_BR2$ polypeptide of claim 18 which comprises:

a. inserting a nucleic acid encoding the $GABA_BR2$ polypeptide into a suitable vector;

b. introducing the resulting vector in a suitable host cell;

c. placing the resulting cell in suitable condition permitting the production of the $GABA_BR2$ polypeptide;

d. recovering the polypeptide produced by the resulting cell; and

e. isolating or purifying the polypeptide so recovered.

58. A $GABA_BR1/R2$ receptor comprising two polypeptides, one of which is a $GABA_BR2$ polypeptide and another of which is a $GABA_BR1$ polypeptide.

59. A method of forming a $GABA_BR1/R2$ receptor which comprises inducing cells to express both a $GABA_BR1$ polypeptide and a $GABA_BR2$ polypeptide.

60. An antibody capable of binding to a $GABA_BR1/R2$ receptor, wherein the $GABA_BR2$ polypeptide is encoded by the nucleic acid of claim 1.

61. The antibody of claim 60, wherein the $GABA_BR2$ polypeptide is a human $GABA_BR2$ polypeptide.

62. An antibody capable of competitively inhibiting the binding of the antibody of claim 60 to a $GABA_BR1/R2$ receptor.

63. An antibody of claim 60, wherein the antibody is a monoclonal antibody.

64. A monoclonal antibody of claim 63 directed to an epitope of a $GABA_BR1/R2$ receptor present on the surface of a $GABA_BR1/R2$ polypeptide expressing cell.

65. A pharmaceutical composition which comprises an amount of the antibody of claim 60 effective to block binding of a ligand to the $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

66. A transgenic, nonhuman mammal expressing a $GABA_BR1/R2$ receptor, which is not naturally expressed by the mammal.

67. A transgenic, nonhuman mammal comprising a homologous recombination knockout of the native $GABA_BR1/R2$ receptor.

68. A transgenic, nonhuman mammal of claim 66 or 67, wherein the transgenic nonhuman mammal is a mouse.

69. A method of detecting the presence of a $GABA_BR1/R2$

receptor on the surface of a cell which comprises contacting the cell with the antibody of claim 60 under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a $GABA_B$R1/R2 receptor on the surface of the cell.

70. A method of determining the physiological effects of varying levels of activity of $GABA_B$R1/R2 receptors which comprises producing a transgenic nonhuman mammal of claim 66 whose levels of $GABA_B$R1/R2 receptor activity vary due to the presence of an inducible promoter which regulates $GABA_B$R1/R2 receptor expression.

71. A method of determining the physiological effects of varying levels of activity of $GABA_B$R1/R2 receptors which comprises producing a panel of transgenic nonhuman mammals of claim 66, each expressing a different amount of $GABA_B$R1/R2 receptor.

72. A method for identifying an antagonist capable of alleviating an abnormality, by decreasing the activity of a $GABA_B$R1/R2 receptor comprising administering a compound to the transgenic nonhuman mammal of claim 66 or 68, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as the antagonist.

73. An antagonist identified by the method of claim 72.

74. A pharmaceutical composition comprising an antagonist of claim 73 and a pharmaceutically acceptable carrier.

75. A method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a $GABA_BR1/R2$ receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 74, thereby treating the abnormality.

76. A method for identifying an agonist capable of alleviating an abnormality, by increasing the activity of a $GABA_BR1/R2$ receptor comprising administering a compound to the transgenic nonhuman mammal of claim 66 or 68, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as the agonist.

77. An agonist identified by the method of claim 76.

78. A pharmaceutical composition comprising an agonist of claim 76 and a pharmaceutically acceptable carrier.

79. A method for treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a $GABA_BR1/R2$ receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 78, thereby treating the abnormality.

80. A cell which expresses on its surface a mammalian $GABA_BR1/R2$ receptor that is not naturally expressed on the surface of such cell.

81. A cell of claim 80, wherein the mammalian $GABA_BR1/R2$ receptor comprises two polypeptides, one of which is a $GABA_BR2$ polypeptide and another of which is a $GABA_BR1$ polypeptide.

82. A process for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor.

83. A process for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises contacting a membrane fraction from a cell extract of cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$

receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor.

84. The process of claim 82 or 83, wherein the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

85. The process of claim 82 or 83, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

86. The process of claim 82 or 83, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same sequence as the amino acid sequence shown in Figures 2A-2D (Seq. ID No. 2).

87. The process of claim 82 or 83, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the amino acid sequence shown in Figures 2A-2D (Seq. ID No. 2).

88. The process of claims 82 or 83, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid TL-267 (ATCC Accession No. 209103).

89. The process of claim 82 or 83, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as the sequence shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

90. The process of claim 82 or 83, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the sequence shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

91. The process of claim 89, wherein the compound is not previously known to bind to a $GABA_BR1/R2$ receptor.

92. A compound identified by the process of claim 91.

93. A process of claim 89, wherein the cell is an insect cell.

94. A process of claim 89, wherein the cell is a mammalian cell.

95. A process of claim 94, wherein the cell is nonneuronal in origin.

96. A process of claim 95, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell a mouse Y1 cell or LM(tk-)

cell.

97. A process of claim 94, wherein the compound is not previously known to bind to a $GABA_BR1/R2$ receptor.

98. A compound identified by the process of claim 97.

99. A process involving competitive binding for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises separately contacting cells expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor, a decrease in the binding of the second chemical compound to the $GABA_BR1/R2$ receptor in the presence of the chemical compound indicating that the chemical compound binds to the $GABA_BR1/R2$ receptor.

100. A process involving competitive binding for identifying a chemical compound which specifically binds to a human $GABA_BR1/R2$ receptor which comprises separately contacting a membrane fraction from a cell extract of cells expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with both the chemical compound and a second chemical

compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor, a decrease in the binding of the second chemical compound to the $GABA_BR1/R2$ receptor in the presence of the chemical compound indicating that the chemical compound binds to the $GABA_BR1/R2$ receptor.

101. A process of claim 99 or 100, wherein the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

102. The process of claim 101, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by plasmid BO-55 (ATCC Accession No. 209104).

103. The process of claim 99 or 100, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in Figures 2A-2D (Seq. ID No. 2).

104. The process of claim 99 or 100, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the amino acid sequence shown in Figures 2A-2D (Seq. ID No. 2).

105. The process of claim 99 or 100, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide

which has substantially the same amino acid sequence as that encoded by plasmid TL-267 (ATCC Accession No. 209103).

106. The process of claim 99 or 100, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as the sequence shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

107. The process of claim 99 or 100, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the sequence shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

108. The process of claim 107, wherein the cell is an insect cell.

109. The process of claim 107, wherein the cell is a mammalian cell.

110. The process of claim 109, wherein the cell is nonneuronal in origin.

111. The process of claim 110, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell a mouse Y1 cell or LM(tk-) cell.

112. The process of claim 109, wherein the compound is not previously known to bind to a $GABA_BR1/R2$ receptor.

113. A compound identified by the process of claim 112.

114. A method of screening a plurality of chemical compounds not known to bind to a $GABA_BR1/R2$ receptor to identify a compound which specifically binds to the $GABA_BR1/R2$ receptor, which comprises

(a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with a compound known to bind specifically to the $GABA_BR1/R2$ receptor;

(b) contacting the same cells as in step (a) with the plurality of compounds not known to bind specifically to the $GABA_BR1/R2$ receptor, under conditions permitting binding of compounds known to bind the $GABA_BR1/R2$ receptor;

(c) determining whether the binding of the compound known to bind specifically to the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of the compounds, relative to the binding of the compound in the absence of the plurality of compounds, and if the binding is reduced;

(d) separately determining the extent of binding to the $GABA_BR1/R2$ receptor of each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which specifically binds to the $GABA_BR1/R2$ receptor.

115. A method of screening a plurality of chemical compounds not known to bind to a $GABA_BR1/R2$ receptor to identify a compound which specifically binds to the $GABA_BR1/R2$ receptor, which comprises

(a) contacting a membrane fraction extract from cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with a compound known to bind specifically to the $GABA_BR1/R2$ receptor;

(b) contacting the same membrane fraction as in step (a) with the plurality of compounds not known to bind specifically to the $GABA_BR1/R2$ receptor, under conditions permitting binding of compounds known to bind the $GABA_BR1/R2$ receptor;

(c) determining whether the binding of the compound known to bind specifically to the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality

of compounds, and if the binding is reduced;

(d) separately determining the extent of binding to the $GABA_BR1/R2$ receptor of each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which specifically binds to the $GABA_BR1/R2$ receptor.

116. A method of claim 114 or 115, wherein the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

117. A method of either of claim 114 or 115, wherein the cell is a mammalian cell.

118. A method of claim 117, wherein the mammalian cell is non-neuronal in origin.

119. The method of claim 118, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell or an NIH-3T3 cell.

120. A process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor agonist which comprises contacting cells with the compound under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting an increase in $GABA_BR1/R2$ receptor activity, so as to thereby

determine whether the compound is a $GABA_BR1/R2$ receptor agonist.

121. A process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor antagonist which comprises contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the compound in the presence of a known $GABA_BR1/R2$ receptor agonist, under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting a decrease in $GABA_BR1/R2$ receptor activity, so as to thereby determine whether the compound is a $GABA_BR1/R2$ receptor antagonist.

122. A process of claim 120 or 121, wherein the cells additionally express nucleic acid encoding GIRK1 and GIRK4.

123. A process of any one of claims 120, 121, or 122, wherein the $GABA_BR2$ receptor is a mammalian $GABA_BR2$ receptor.

124. A pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor agonist determined to be an agonist by the process of claim 120 effective to increase activity of a $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

125. A pharmaceutical composition of claim 124, wherein

the $GABA_BR1/R2$ receptor agonist was not previously known.

126. A pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor antagonist determined to be an antagonist the process of claim 121 effective to reduce activity of a $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

127. A pharmaceutical composition of claim 126, wherein the $GABA_BR1/R2$ receptor antagonist was not previously known.

128. A process for determining whether a chemical compound activates a $GABA_BR1/R2$ receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the chemical compound under conditions suitable for activation of the $GABA_BR1/R2$ receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the $GABA_BR1/R2$ receptor.

129. The process of claim 128, wherein the second messenger response comprises potassium channel activation and the change in second messenger is an increase in the level of potassium current.

130. A process for determining whether a chemical compound inhibits activation of a $GABA_BR1/R2$ receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with both the chemical compound and a second chemical compound known to activate the $GABA_BR1/R2$ receptor, and with only the second chemical compound, under conditions suitable for activation of the $GABA_BR1/R2$ receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the $GABA_BR1/R2$ receptor.

131. The process of claim 130, wherein the second messenger response comprises potassium channel activation and the change in second messenger response is a smaller increase in the level of inward potassium current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

132. A process of any one of claims 128, 129, 130 or 131, wherein the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

133. The process of claim 132, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

134. The process of claim 132, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in Figures 4A-4D (Seq. ID No. 4).

135. The process of claim 132, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

136. The process of claim 132, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the sequence, shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

137. The process of claim 132, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid TL-267 (ATCC Accession No. 209103).

138. The process of any one of claims 128-131, wherein the cell is an insect cell.

139. The process of any one of claims 128-131, wherein the cell is a mammalian cell.

140. The process of claim 139, wherein the mammalian cell is nonneuronal in origin.

141. The process of claim 140, wherein the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

142. The process of claim 139, wherein the compound was not previously known to activate or inhibit a $GABA_BR1/R2$ receptor.

143. A compound determined by the process of claim 142.

144. A pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor agonist determined by the process of claim 128 or 129 effective to increase activity of a $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

145. A pharmaceutical composition of claim 144, wherein the $GABA_BR1/R2$ receptor agonist was not previously known.

146. A pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor antagonist determined

by the process of claim 130 or 131 effective to reduce activity of a $GABA_B$R1/R2 receptor and a pharmaceutically acceptable carrier.

147. A pharmaceutical composition of claim 146, wherein the $GABA_B$R1/R2 receptor antagonist was not previously known.

148. A method of screening a plurality of chemical compounds not known to activate a $GABA_B$R1/R2 receptor to identify a compound which activates the $GABA_B$R1/R2 receptor which comprises:

(a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_B$R1/R2 receptor, wherein such cells do not normally express the $GABA_B$R1/R2 receptor, with the plurality of compounds not known to activate the $GABA_B$R1/R2 receptor, under conditions permitting activation of the $GABA_B$R1/R2 receptor;

(b) determining whether the activity of the $GABA_B$R1/R2 receptor is increased in the presence of the compounds, and if it is increased;

(c) separately determining whether the activation of the $GABA_B$R1/R2 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the

compound or compounds present in such plurality of compounds which activates the $GABA_BR1/R2$ receptor.

149. The process of claim 148, wherein the cells express nucleic acid encoding GIRK1 and GIRK4.

150. A method of claim 148 or 149, wherein the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

151. A method of screening a plurality of chemical compounds not known to inhibit the activation of a $GABA_BR1/R2$ receptor to identify a compound which inhibits the activation of the $GABA_BR1/R2$ receptor, which comprises:

(a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the plurality of compounds in the presence of a known $GABA_BR1/R2$ receptor agonist, under conditions permitting activation of the $GABA_BR1/R2$ receptor;

(b) determining whether the activation of the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of compounds, relative to the activation of the $GABA_BR1/R2$ receptor in the absence of the plurality of compounds, and if it is reduced;

(c) separately determining the inhibition of activation of the $GABA_BR1/R2$ receptor for each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such a plurality of compounds which inhibits the activation of the $GABA_BR1/R2$ receptor.

152. The process of claim 151, wherein the cells express nucleic acid encoding GIRK1 and GIRK4.

153. A method of claim 151 or 152, wherein the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

154. A method of any one of claims 148, 149, 151, or 152, wherein the cell is a mammalian cell.

155. A method of claim 154, wherein the mammalian cell is non-neuronal in origin.

156. The method of claim 155, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell or an NIH-3T3 cell.

157. A pharmaceutical composition comprising a compound identified by the method of claim 148 or 149, effective to increase $GABA_BR1/R2$ receptor activity and a pharmaceutically acceptable carrier.

158. A pharmaceutical composition comprising a compound identified by the method of claim 151 or 152, effective to decrease $GABA_B$R1/R2 receptor activity and a pharmaceutically acceptable carrier.

159. A process for determining whether a chemical compound is a $GABA_B$R1/R2 receptor agonist, which comprises preparing a membrane fraction from cells which comprise nucleic acid encoding and expressing on their cell surface the $GABA_B$R1/R2 receptor, wherein such cells do not normally express the $GABA_B$R1/R2 receptor, separately contacting the membrane fraction with both the chemical compound and GTPγS, and with only GTPγS, under conditions permitting the activation of the $GABA_B$R1/R2 receptor, and detecting GTPγS binding to the membrane fraction, an increase in GTPγS binding in the presence of the compound indicating that the chemical compound activates the $GABA_B$R1/R2 receptor.

160. A process for determining whether a chemical compound is a $GABA_B$R1/R2 receptor antagonist, which comprises preparing a membrane fraction from cells which comprise nucleic acid encoding and expressing on their cell surface the $GABA_B$R1/R2 receptor, wherein such cells do not normally express the $GABA_B$R1/R2 receptor, separately contacting the membrane fraction with the chemical compound, GTPγS and a second chemical compound known to activate the $GABA_B$R1/R2 receptor, with GTPγS and only the second compound, and with GTPγS alone, under conditions permitting the activation of the $GABA_B$R1/R2 receptor, detecting GTPγS binding to each membrane fraction, and comparing the increase in GTPγS binding in the presence of the compound and the second compound

relative to the binding of GTPγS alone, to the increase in GTPγS binding in the presence of the second chemical compound known to activate the $GABA_BR1/R2$ receptor relative to the binding of GTPγS alone, a smaller increase in GTPγS binding in the presence of the compound and the second compound indicating that the compound is a $GABA_BR1/R2$ receptor antagonist.

161. A process of claim 159 or 160, wherein the $GABA_BR2$ receptor is a mammalian $GABA_BR2$ receptor.

162. The process of claim 161, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

163. The process of claim 162, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in Figures 4A-4D (Seq. ID No. 4).

164. The process of claim 161, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid TL-267 (ATCC Accession No. 209103).

165. The process of claim 161, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has

substantially the same amino acid sequence as that shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

166. The process of claim 161, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the sequence shown in Figures 2A-2D (Seq. ID No. 2) from amino acid 19 through amino acid 898.

167. The process of claim 159 or 160, wherein the cell is an insect cell.

168. The process of claim 159 or 160, wherein the cell is a mammalian cell.

169. The process of claim 168, wherein the mammalian cell is nonneuronal in origin.

170. The process of claim 169, wherein the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

171. The process of claim 170, wherein the compound was not previously known to be an agonist or antagonist of a $GABA_BR1/R2$ receptor.

172. A compound determined to be an agonist or antagonist of a $GABA_BR1/R2$ receptor by the process of claim 171.

173. A method of treating spasticity in a subject which comprises administering to the subject an amount of a compound which is an agonist of a $GABA_BR1/R2$ receptor effective to treat spasticity in the subject.

174. A method of treating asthma in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to treat asthma in the subject.

175. A method of treating incontinence in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to treat incontinence in the subject.

176. A method of decreasing nociception in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to decrease nociception in the subject.

177. A use of a $GABA_BR2$ agonist as an antitussive agent which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective as an antitussive agent in the subject.

178. A method of treating drug addiction in a subject which comprises administering to the subject an

amount of a compound which is a $GABA_B$R1/R2 receptor agonist effective to treat drug addiction in the subject.

179. A method of treating Alzheimer's disease in a subject which comprises administering to the subject an amount of a compound which is a $GABA_B$R1/R2 receptor antagonist effective to treat Alzheimer's disease in the subject.

182. A process for making a composition of matter which specifically binds to a $GABA_B$R1/R2 receptor which comprises identifying a chemical compound using the process af any of claims, 82, 83, 99, 100, 114 or 115 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

183. A process for making a composition of matter which specifically binds to a $GABA_B$R1/R2 receptor which comprises identifying a chemical compound using the process of any of claims 120, 128, or 148 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

184. A process for making a composition of matter which specifically binds to a $GABA_B$R1/R2 receptor which comprises identifying a chemical compound using the process of any of claims 121, 130, or 151 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

185. The process of any of claims 182, 183, or 184, wherein the $GABA_B$R1/R2 receptor is a human $GABA_B$R1/R2 receptor.

186. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by the process of any of claims 82, 83, 99, 100, 114 or 115

or a novel structural and functional analog or homolog thereof.

187. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by the process of any of claims 120, 128, or 148 or a novel structural and functional analog or homolog thereof.

188. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by the process of any of claims 121, 130, or 151 or a novel structural and functional analog or homolog thereof.

189. The process of any of claims 186, 187, or 188, wherein the $GABA_BR1/R2$ receptor is a human $GABA_BR1/R2$ receptor.

PCT INTERNATIONAL APPLICATION TRANSMITTAL LETTER	DATE: 16 October 1998
REGARDING THE INTERNATIONAL APPLICATION OF SYNAPTIC PHARMACEUTICAL CORPORATION	DOCKET OR REFERENCE NUMBER 54002-B-PCT/JPW/ADM

ENTITLED

DNA ENCODING A $GABA_BR2$ POLYPEPTIDE AND USES THEREOF

Certification under 37 CFR 1.10 (if applicable)

EM 525 892 727 US — "Express Mail" mailing number

16 October 1998 — Date of Deposit

I hereby certify that this application is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Jessica Mentzavanis — (Typed or printed name of person mailing application)

[signature] — (Signature of person mailing application)

To the United States Receiving Office (RO/US):

Accompanying this transmittal letter is the above-identified International application, including a completed Request form (PCT/RO/101). Please process the application according to the provisions of the Patent Cooperation Treaty.

The following requests are made of the RO/US:

1. [X] PREPARATION AND TRANSMITTAL OF CERTIFIED COPY OF PRIORITY DOCUMENTS--Please prepare and transmit to the International Bureau a certified copy of the United States origin priority documents identified in Box VI of the Request form (37 CFR 1.451).

   To cover the cost of copy preparation and certification (37 CFR 1.19(a)(3) and (b)(1)),

   [ ] a (check) (money order) in the amount of $________ is attached to this transmittal letter.

   [X] the RO/US is hereby authorized to charge the following deposit account no.: 03-3125

   The appropriate Search fee for the above-named Authority is indicated on the Fee Calculation Sheet (PCT/RO/101 Annex).

2. [X] SUPPLEMENTAL SEARCH FEES (ONLY WHEN ISA/US CONDUCTS THE INTERNATIONAL SEARCH.)—Please charge any Supplemental Search fees that may be required by the United States International Searching Authority (ISA/US) to deposit account no.: 03-3125

   I understand that this authorization is subject to my oral confirmation thereof in each instance and that it in no way limits my right to submit a protest against payment of the Supplemental Search fees, but is merely an administrative aid to assure that the ISA/US may timely complete the Search Report.

NOTE: SUPPLEMENTAL SEARCH FEES FOR ISA/EP ARE PAYABLE DIRECTLY TO THE EUROPEAN PATENT OFFICE

3. [X] DISCLOSURE INFORMATION—In order to assist in screening the accompanying International application for purposes of determining whether a license for foreign transmittal should and could be granted, the following information is supplied:

   A. [ ] There is no prior filed application relating to this invention.

   B. [X] There is a prior application*, serial number 08/953,277 and 09/141,760 filed on 17 October 1997 27 August 1998 which contains subject matter that is

      1. [ ] substantially identical to that of the accompanying International application.
      2. [X] less than that of the accompanying International application. The additional subject matter of the International application appears on pages(s) and line(s) See Attachment A.
      3. [ ] more than that of the accompanying International application.

   C. [ ] Disclosure information cannot be covered by the language of Points 4A or 4B above due to the involvement of several prior applications or for other reasons. A separate sheet on which the disclosure information is explained is attached to this transmittal letter.

4. [X] REQUEST FOR FOREIGN TRANSMITTAL LICENSE—According to the provisions of 35 U.S.C. 184 and 37 CFR 5.11, a license to transmit the accompanying International application to foreign agencies or international authorities is hereby requested.

* Priority is not claimed, unless all necessary information is listed in Box VI of the Request Form (PCT/RO/101).

SIGNER IS THE	NAME OF SIGNER (typed)
[ ] APPLICANT [ ] COMMON REPRESENTATIVE [X] (ATTORNEY)(AGENT) REG NO 28,678	John P. White SIGNATURE [signature]

PTO-1382 (REV. 4-90)

U.S. Department of Commerce
Patent and Trademark Office

ATTACHMENT A

Supplemental Sheet further to PCT Transmittal Letter, item 3 subpart B2.:

Page 1: lines 4-6
Page 18: line 31 to page 19
Page 20: lines 30-31
Page 22: lines 28-31
Page 24: line 24 to page 25
Page 26: lines 11-12
Page 60: line 12 to page 62
Page 80: line 13 to page 81
Page 101: line 25 to page 102 line 20
Page 102: lines 28-30
Page 102: line 32 to page 104 line 4
Page 104: lines 19-30
Page 106: lines 15-20
Page 106: line 10
Page 126 lines 11-13
Page 127: lines 20-23
claims 182-189
Figures 20 and 21
Sequence ID Nos. 36 and 37

FIGURE 1A

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-243  TGACCTCGGGGCAGGTCCTGGTGCAGAGCGTCGCCAAGGACGCCGAGAGGGAGGCGGGAT  -184
-183  TGCCCAGACATCCTTCAGCGAAGTGCATGTGTGTTTGTAAACCATCGTTGGCTGTCGGGA  -124
-123  GACCGCGAGGACCGGTCCAGGCTGCGGCGGAGTCGAGGGCGAGGGAGAGGCCGCGTGAGT   -64
 -63  GAGCAGAGTCCAGAGCCGTGCGCCCCCAGAACTGCGCGTCCGCCCCGTGCACCCCCGCGC    -4
  -3  GCCATGCCCAGTTGCCCCGCGCGCTCTGCTACGGGCCCGCTCTCCATCATGGGCCTCATG    57
  58  CCGCTCACCAAGGAGGTGGCCAAGGGCAGCATCGGGCGCGGTGTGCTCCCCGCCGTGGAA   117
 118  CTGGCCATCGAGCAGATCCGCAACGAGTCACTCCTGCGCCCCTACTTCCTCGACCTGCGG   177
 178  CTCTATGACACGGAGTGCGACAACGCAAAAGGGTTGAAAGCCTTCTACGATGCGATAAAA   237
 238  TACGGGCCGAACCACTTGATGGTGTTTGGAGGCGTCTGTCCATCCGTCACATCCATCATT   297
 298  GCAGAGTCCCTCCAAGGCTGGAATCTGGTGCAGCTTTCTTTTGCTGCAACCACGCCTGTT   357
 358  CTAGCCGATAAGAAAAAATACCCTTATTTCTTTCGGACCGTCCCATCAGACAATGCGGTG   417
 418  AATCCAGCCATTCTGAAGTTGCTCAAGCACTACCAGTGGAAGCGCGTGGGCACGCTGACG   477
 478  CAAGACGTTCAGAGGTTCTCTGAGGTGCGGAATGACCTGACTGGAGTTCTGTATGGCGAG   537
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FIGURE 1B

538 GACATTGAGATTTCAGACACCGAGAGCTTCTCCAACGATCCCTGTACCAGTGTCAAAAAG 597
598 CTGAAGGGGAATGATGTGCGGATCATCCTTGGCCAGTTTGACCAGAATATGGCAGCAAAA 657
658 GTGTTCTGTTGTGCATACGAGGAGAACATGTATGGTAGTAAATATCAGTGGATCATTCCG 717
718 GGCTGGTACGAGCCTTCTTGGTGGAGCAGGTGCACACGGAAGCCAACTCATCCCGCTGC 777
778 CTCCGGAAGAATCTGCTTGCTGCCATGGAGGGCTACATTGGCGTGGATTTCGAGCCCCTG 837
838 AGCTCCAAGCAGATCAAGACCATCTCAGGAAAGACTCCACAGCAGTATGAGAGAGAGTAC 897
898 AACAACAAGCGGTCAGGCGTGGGGCCCAGCAAGTTCCACGGGTACGCCTACGATGGCATC 957
958 TGGGTCATCGCCAAGACACTGCAGAGGGCCATGGAGACACTGCATGCCAGCAGCCGGCAC 1017
1018 CAGCGGATCCAGGACTTCAACTACACGGACCACACGCTGGGCAGGATCATCCTCAATGCC 1077
1078 ATGAACGAGACCAACTTCTTCGGGGTCACGGGTCAAGTTGTATTCCGGAATGGGGAGAGA 1137
1138 ATGGGGACCATTAAATTTACTCAATTTCAAGACAGCAGGGAGGTGAAGGTGGGAGAGTAC 1197
1198 AACGCTGTGGCCGACACACTGGAGATCATCAATGACACCATCAGGTTCCAAGGATCCGAA 1257
1258 CCACCAAAAGACAAGACCATCATCCTGGAGCAGCTGCGGAAGATCTCCCTACCTCTCTAC 1317

FIGURE 1C

1318 AGCATCCTCTCTGCCCTCACCATCCTCGGGATGATCATGGCCAGTGCTTTTCTCTTCTTC 1377
1378 AACATCAAGAACCGGAATCAGAAGCTCATAAAGATGTCGAGTCCATACATGAACAACCTT 1437
1438 ATCATCCTTGGAGGGATGCTTTCCTATGCTTCCATATTTCTCTTTGGCCTTGATGGATCC 1497
1498 TTTGTCTCTGAAAAGACCTTTGAAACACTTTGCACCGTCAGGACCTGGATTCTCACCGTG 1557
1558 GGCTACACGACCGCTTTTGGGGCCATGTTTGCAAAGACCTGGAGAGTCCACGCCATCTTC 1617
1618 AAAAATGTGAAAATGAAGAAGAAGATCATCAAGGACCAGAAACTGCTTGTGATCGTGGGG 1677
1678 GGCATGCTGCTGATCGACCTGTGTATCCTGATCTGCTGGCAGGCTGTGGACCCCCTGCGA 1737
1738 AGGACAGTGGAGAAGTACAGCATGGAGCCGGACCCAGCAGGACGGGATATCTCCATCCGC 1797
1798 CCTCTCCTGGAGCACTGTGAGAACACCCATATGACCATCTGGCTTGGCATCGTCTATGCC 1857
1858 TACAAGGGACTTCTCATGTTGTTCGGTTGTTTCTTAGCTTGGGAGACCCGCAACGTCAGC 1917
1918 ATCCCCGCACTCAACGACAGCAAGTACATCGGGATGAGTGTCTACAACGTGGGGATCATG 1977
1978 TGCATCATCGGGGCCGCTGTCTCCTTCCTGACCCGGGACCAGCCCAATGTGCAGTTCTGC 2037
2038 ATCGTGGCTCTGGTCATCATCTTCTGCAGCACCATCACCCTCTGCCTGGTATTCGTGCCG 2097

FIGURE 1D

2098 AAGCTCATCACCCTGAGAACAAACCCAGATGCAGCAACGCAGAACAGGCGATTCCAGTTC 2157
2158 ACTCAGAATCAGAAGAAAGAAGATTCTAAAACGTCCACCTCGGTCACCAGTGTGAACCAA 2217
2218 GCCAGCACATCCCGCCTGGAGGGCCTACAGTCAGAAAACCATCGCCTGCGAATGAAGATC 2277
2278 ACAGAGCTGGATAAAGACTTGGAAGAGGTCACCATGCAGCTGCAGGACACACCAGAAAAG 2337
2338 ACCACCTACATTAAACAGAACCACTACCAAGAGCTCAATGACATCCTCAACCTGGGAAAC 2397
2398 TTCACTGAGAGCACAGATGGAGGAAAGGCCATTTTAAAAAATCACCTCGATCAAAATCCC 2457
2458 CAGCTACAGTGGAACACAACAGAGCCCTCTCGAACATGCAAAGATCCTATAGAAGATATA 2517
2518 AACTCTCCAGAACACATCCAGCGTCGGCTGTCCCTCCAGCTCCCCATCCTCCACCACGCC 2577
2578 TACCTCCCATCCATCGGAGGCGTGGACGCCAGCTGTGTCAGCCCCTGCGTCAGCCCCACC 2637
2638 GCCAGCCCCCGCCACAGACATGTGCCACCCTCCTTCCGAGTCATGGTCTCGGGCCTGTAA 2697
2698 GGGTGGGAGGCCTGGGCCCGGGGCCTCCCCCGTGACAGAACCACACTGGGCAGAGGGGTC 2757
2758 TGCTGCAGAAACACTGTCGGCTCTGGCTGCGGAGAAGCTGGGCACCATGGCTGGCCTCTC 2817
2818 AGGACCACTCGGATGGCACTCAGGTGGACAGGACGGGGCAGGGGGAGACTTGGCACCTGA 2877

FIGURE 1E

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2878  CCTCGAGCCTTATTTGTGAAGTCCTTATTTCTTCACAAAGAAGAGGAACGGAAATGGGAC  2937
2938  GTCTTCCTTAACATCTGCAAACAAGGAGGCGCTGGGATATCAAACTTGCAAAAAAAAAAA  2997
2998  AAAA                                                          3001
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182. A process for making a composition of matter which specifically binds to a $GABA_BR1/R2$ receptor which comprises identifying a chemical compound using the process af any of claims, 82, 83, 99, 100, 114 or 115 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof..

183. A process for making a composition of matter which specifically binds to a $GABA_BR1/R2$ receptor which comprises identifying a chemical compound using the process of any of claims 120, 128, or 148 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

184. A process for making a composition of matter which specifically binds to a $GABA_BR1/R2$ receptor which comprises identifying a chemical compound using the process of any of claims 121, 130, or 151 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

185. The process of any of claims 182, 183, or 184, wherein the $GABA_BR1/R2$ receptor is a human $GABA_BR1/R2$ receptor.

186. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by the process of any of claims 82, 83, 99, 100, 114 or 115

or a novel structural and functional analog or homolog thereof.

187. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by the process of any of claims 120, 128, or 148 or a novel structural and functional analog or homolog thereof.

188. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by the process of any of claims 121, 130, or 151 or a novel structural and functional analog or homolog thereof.

189. The process of any of claims 186, 187, or 188, wherein the $GABA_BR1/R2$ receptor is a human $GABA_BR1/R2$ receptor.

PCT INTERNATIONAL APPLICATION TRANSMITTAL LETTER	DATE: 16 October 1998
REGARDING THE INTERNATIONAL APPLICATION OF: SYNAPTIC PHARMACEUTICAL CORPORATION	DOCKET OR REFERENCE NUMBER 54002-B-PCT/JPW/ADM

ENTITLED

DNA ENCODING A $GABA_BR2$ POLYPEPTIDE AND USES THEREOF

Certification under 37 CFR 1.10 (if applicable)

EM 525 892 727 US — "Express Mail" mailing number

16 October 1998 — Date of Deposit

I hereby certify that this application is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Jessica Mantzavinis — (Typed or printed name of person mailing application)

(Signature of person mailing application)

To the United States Receiving Office (RO/US):

Accompanying this transmittal letter is the above-identified International application, including a completed Request form (PCT/RO/101). Please process the application according to the provisions of the Patent Cooperation Treaty.

The following requests are made of the RO/US:

1. [X] PREPARATION AND TRANSMITTAL OF CERTIFIED COPY OF PRIORITY DOCUMENTS—Please prepare and transmit to the International Bureau a certified copy of the United States origin priority documents identified in Box VI of the Request form (37 CFR 1.451).

   To cover the cost of copy preparation and certification (37 CFR 1.19(a)(3) and (b)(1)),
   - [ ] a (check) (money order) in the amount of $________ is attached to this transmittal letter.
   - [X] the RO/US is hereby authorized to charge the following deposit account no.: 03-3125

   The appropriate Search fee for the above-named Authority is indicated on the Fee Calculation Sheet (PCT/RO/101 Annex).

2. [X] SUPPLEMENTAL SEARCH FEES (ONLY WHEN ISA/US CONDUCTS THE INTERNATIONAL SEARCH.)—Please charge any Supplemental Search fees that may be required by the United States International Searching Authority (ISA/US) to deposit account no.: 03-3125

   I understand that this authorization is subject to my oral confirmation thereof in each instance and that it in no way limits my right to submit a protest against payment of the Supplemental Search fees, but is merely an administrative aid to assure that the ISA/US may timely complete the Search Report.

NOTE: SUPPLEMENTAL SEARCH FEES FOR ISA/EP ARE PAYABLE DIRECTLY TO THE EUROPEAN PATENT OFFICE

3. [X] DISCLOSURE INFORMATION—In order to assist in screening the accompanying International application for purposes of determining whether a license for foreign transmittal should and could be granted, the following information is supplied:

   A. [ ] There is no prior filed application relating to this invention.

   B. [X] There is a prior application*, serial number 08/953,277 and 09/141,760 filed on 17 October 1997 27 August 1998 which contains subject matter that is
      1. [ ] substantially identical to that of the accompanying International application.
      2. [X] less than that of the accompanying International application. The additional subject matter of the International application appears on pages(s) and line(s) See Attachment A.
      3. [ ] more than that of the accompanying International application.

   C. [ ] Disclosure information cannot be covered by the language of Points 4A or 4B above due to the involvement of several prior applications or for other reasons. A separate sheet on which the disclosure information is explained is attached to this transmittal letter.

4. [X] REQUEST FOR FOREIGN TRANSMITTAL LICENSE—According to the provisions of 35 U.S.C. 184 and 37 CFR 5.11, a license to transmit the accompanying International application to foreign agencies or international authorities is hereby requested.

* Priority is not claimed, unless all necessary information is listed in Box VI of the Request Form (PCT/RO/101).

SIGNER IS THE	NAME OF SIGNER (typed)
[ ] APPLICANT [ ] COMMON REPRESENTATIVE [X] (ATTORNEY/AGENT) REG NO 28,678	John P. White SIGNATURE: John P White

PTO-1382 (REV. 4-90) — U.S. Department of Commerce, Patent and Trademark Office

ATTACHMENT A

Supplemental Sheet further to PCT Transmittal Letter, item 3 subpart B2.:

Page 1: lines 4-6
Page 18: line 31 to page 19
Page 20: lines 30-31
Page 22: lines 28-31
Page 24: line 24 to page 25
Page 26: lines 11-12
Page 60: line 12 to page 62
Page 80: line 13 to page 81
Page 101: line 25 to page 102 line 20
Page 102: lines 28-30
Page 102: line 32 to page 104 line 4
Page 104: lines 19-30
Page 106: lines 15-20
Page 106: line 10
Page 126 lines 11-13
Page 127: lines 20-23
claims 182-189
Figures 20 and 21
Sequence ID Nos. 36 and 37

FIGURE 1A

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-243  TGACCTCGGGGCAGGTCCTGGTGCAGAGCGTCGCCAAGGACGCCGAGAGGGAGGCGGGAT  -184
-183  TGCCCAGACATCCTTCAGCGAAGTGCATGTGTGTTTGTAAACCATCGTTGGCTGTCGGGA  -124
-123  GACCGCGAGGACCGGTCCAGGCTGCGGCGGAGTCGAGGGCGAGGGAGAGGCCGCGTGAGT   -64
 -63  GAGCAGAGTCCAGAGCCGTGCGCCCCCAGAACTGCGCGTCCGCCCCGTGCACCCCCGCGC    -4
  -3  GCCATGCCCAGTTGCCCCGCGCGCTCTGCTACGGGCCCGCTCTCCATCATGGGCCTCATG    57
  58  CCGCTCACCAAGGAGGTGGCCAAGGGCAGCATCGGGCGCGGTGTGCTCCCCGCCGTGGAA   117
 118  CTGGCCATCGAGCAGATCCGCAACGAGTCACTCCTGCGCCCCTACTTCCTCGACCTGCGG   177
 178  CTCTATGACACGGAGTGCGACAACGCAAAAGGGTTGAAAGCCTTCTACGATGCGATAAAA   237
 238  TACGGGCCGAACCACTTGATGGTGTTTGGAGGCGTCTGTCCATCCGTCACATCCATCATT   297
 298  GCAGAGTCCCTCCAAGGCTGGAATCTGGTGCAGCTTTCTTTTGCTGCAACCACGCCTGTT   357
 358  CTAGCCGATAAGAAAAAATACCCTTATTTCTTTCGGACCGTCCCATCAGACAATGCGGTG   417
 418  AATCCAGCCATTCTGAAGTTGCTCAAGCACTACCAGTGGAAGCGCGTGGGCACGCTGACG   477
 478  CAAGACGTTCAGAGGTTCTCTGAGGTGCGGAATGACCTGACTGGAGTTCTGTATGGCGAG   537
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FIGURE 1B

538 GACATTGAGATTTCAGACACCGAGAGCTTCTCCAACGATCCCTGTACCAGTGTCAAAAAG 597
598 CTGAAGGGGAATGATGTGCGGATCATCCTTGGCCAGTTTGACCAGAATATGGCAGCAAAA 657
658 GTGTTCTGTTGTGCATACGAGGAGAACATGTATGGTAGTAAATATCAGTGGATCATTCCG 717
718 GGCTGGTACGAGCCTTCTTGGTGGGAGCAGGTGCACACGGAAGCCAACTCATCCCGCTGC 777
778 CTCCGGAAGAATCTGCTTGCTGCCATGGAGGGCTACATTGGCGTGGATTTCGAGCCCCTG 837
838 AGCTCCAAGCAGATCAAGACCATCTCAGGAAAGACTCCACAGCAGTATGAGAGAGAGTAC 897
898 AACAACAAGCGGTCAGGCGTGGGGCCCAGCAAGTTCCACGGGTACGCCTACGATGGCATC 957
958 TGGGTCATCGCCAAGACACTGCAGAGGGCCATGGAGACACTGCATGCCAGCAGCCGGCAC 1017
1018 CAGCGGATCCAGGACTTCAACTACACGGACCACACGCTGGGCAGGATCATCCTCAATGCC 1077
1078 ATGAACGAGACCAACTTCTTCGGGGTCACGGGTCAAGTTGTATTCCGGAATGGGGAGAGA 1137
1138 ATGGGGACCATTAAATTTACTCAATTTCAAGACAGCAGGGAGGTGAAGGTGGGAGAGTAC 1197
1198 AACGCTGTGGCCGACACACTGGAGATCATCAATGACACCATCAGGTTCCAAGGATCCGAA 1257
1258 CCACCAAAAGACAAGACCATCATCCTGGAGCAGCTGCGGAAGATCTCCCTACCTCTCTAC 1317

FIGURE 1C

1318	AGCATCCTCTCTGCCCTCACCATCCTCGGGATGATCATGGCCAGTGCTTTTCTCTTCTTC	1377
1378	AACATCAAGAACCGGAATCAGAAGCTCATAAAGATGTCGAGTCCATACATGAACAACCTT	1437
1438	ATCATCCTTGGAGGGATGCTTTCCTATGCTTCCATATTTCTCTTTGGCCTTGATGGATCC	1497
1498	TTTGTCTCTGAAAAGACCTTTGAAACACTTTGCACCGTCAGGACCTGGATTCTCACCGTG	1557
1558	GGCTACACGACCGCTTTTGGGGCCATGTTTGCAAAGACCTGGAGAGTCCACGCCATCTTC	1617
1618	AAAAATGTGAAAATGAAGAAGAAGATCATCAAGGACCAGAAACTGCTTGTGATCGTGGGG	1677
1678	GGCATGCTGCTGATCGACCTGTGTATCCTGATCTGCTGGCAGGCTGTGGACCCCCTGCGA	1737
1738	AGGACAGTGGAGAAGTACAGCATGGAGCCGGACCCAGCAGGACGGGATATCTCCATCCGC	1797
1798	CCTCTCCTGGAGCACTGTGAGAACACCCATATGACCATCTGGCTTGGCATCGTCTATGCC	1857
1858	TACAAGGGACTTCTCATGTTGTTCGGTTGTTTCTTAGCTTGGGAGACCCGCAACGTCAGC	1917
1918	ATCCCCGCACTCAACGACAGCAAGTACATCGGGATGAGTGTCTACAACGTGGGGATCATG	1977
1978	TGCATCATCGGGGCCGCTGTCTCCTTCCTGACCCGGGACCAGCCCAATGTGCAGTTCTGC	2037
2038	ATCGTGGCTCTGGTCATCATCTTCTGCAGCACCATCACCCTCTGCCTGGTATTCGTGCCG	2097

FIGURE 1D

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2098 AAGCTCATCACCCTGAGAACAAACCCAGATGCAGCAACGCAGAACAGGCGATTCCAGTTC 2157
2158 ACTCAGAATCAGAAGAAAGAAGATTCTAAAACGTCCACCTCGGTCACCAGTGTGAACCAA 2217
2218 GCCAGCACATCCCGCCTGGAGGGCCTACAGTCAGAAAACCATCGCCTGCGAATGAAGATC 2277
2278 ACAGAGCTGGATAAAGACTTGGAAGAGGTCACCATGCAGCTGCAGGACACACCAGAAAAG 2337
2338 ACCACCTACATTAAACAGAACCACTACCAAGAGCTCAATGACATCCTCAACCTGGGAAAC 2397
2398 TTCACTGAGAGCACAGATGGAGGAAAGGCCATTTTAAAAAATCACCTCGATCAAAATCCC 2457
2458 CAGCTACAGTGGAACACAACAGAGCCCTCTCGAACATGCAAAGATCCTATAGAAGATATA 2517
2518 AACTCTCCAGAACACATCCAGCGTCGGCTGTCCCTCCAGCTCCCCATCCTCCACCACGCC 2577
2578 TACCTCCCATCCATCGGAGGCGTGGACGCCAGCTGTGTCAGCCCCTGCGTCAGCCCCACC 2637
2638 GCCAGCCCCCGCCACAGACATGTGCCACCCTCCTTCCGAGTCATGGTCTCGGCCTGTAA 2697
2698 GGGTGGGAGGCCTGGGCCCGGGGCCTCCCCCGTGACAGAACCACACTGGGCAGAGGGGTC 2757
2758 TGCTGCAGAAACACTGTCGGCTCTGGCTGCGGAGAAGCTGGGCACCATGGCTGGCCTCTC 2817
2818 AGGACCACTCGGATGGCACTCAGGTGGACAGGACGGGGCAGGGGGAGACTTGGCACCTGA 2877
```

FIGURE 1E

2878	CCTCGAGCCTTATTTGTGAAGTCCTTATTTCTTCACAAAGAAGAGGAACGGAAATGGGAC	2937
2938	GTCTTCCTTAACATCTGCAAACAAGGAGGCGCTGGGATATCAAACTTGCAAAAAAAAAAA	2997
2998	AAAA	3001

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/22033

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/10, 15/12, 5/10; C07K 14/705; C12Q 1/68
US CL : 435/69.1, 6, 320.1, 252.3, 254.11, 325; 530/350; 536/23.1, 23.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 6, 320.1, 252.3, 254.11, 325; 530/350; 536/23.1, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, WPIDS, CAPLUS, GENBANK
search terms: gabab?, gaba?, receptor?, jones k?, laz t?, borowsky b

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KAUPMANN ET AL. Expression cloning of GABAB receptors uncovers similarity to metabotropic glutamate receptors. Nature. 20 March 1997. Vol. 386. pages 239-246, see entire document.	1-37, 57, 58
A	TANAKA ET AL. Desensitization of GABAB receptor expressed in Xenopus oocytes. Pharmacol. Comm. 1992. Vol. 2. Nos. 1-2. pages 20-22, entire document.	1-37, 57, 58
A	BOWERY ET AL. Metabotropic GABAB receptors cloned at last. Trends In Pharm. April 1997. Vol. 18. No. 4. page 103. see entire document.	1-37, 57, 58
A	KERR ET AL. GABAB receptors. Pharmacol. Ther. 1995. Vol. 67. No. 2. pages 187-246, entire document.	1-37, 57, 58

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
06 JANUARY 1999	10 FEB 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CLAIRE M. KAUFMAN Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GENEXPRESS, GenBank Database, National Library of Medicine, Bethesda, Maryland, USA, accession Number Z43654, H. sapiens partial cDNA sequence; clone c-1hh04, 21 September 1995, see entire abstract.	1-37, 57, 58
A	ADAMS ET AL., GenBank Database, National Library of Medicine, Bethesda, Maryland, USA, accession Number T07621, EST 05511 Homo sapiens cDNA clone HFBEL81, 30 June 1993, see entire abstract.	1-37, 57, 58
A	INVITROGEN CORPORATION. Invitrogen Product Catalog 1996. San Diego, California: Invitrogen Corp. and Oxformd & Drozda. 1996, page 26, 30, 31 and 36, see entire abstract.	19-29, 57

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-37, 57, 58

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-37, 57 and 58, drawn to nucleic acid encoding GABABR2, vector, host cell, protein, and method of detecting encoding nucleic acid.
Group II, claim(s) 38, 39, 40, 46, and 47, drawn of inhibiting translation.
Group III, claim(s) 41-45 and 48, drawn to an antibody to GABABR2.
Group IV, claim(s) 49, 52-54, 66, 68, 70-71, drawn to a transgenic expressing the receptor.
Group V, claim(s) 50, 52-54, 67, 68, drawn to a knock-out transgenic.
Group VI, claim(s) 51, 54, drawn to transgenic with antisense nucleic acid.
Group VII, claim(s) 55, drawn to method of detecting GABABR2 with an antibody.
Group VIII, claim(s) 56 and 59, drawn to method of making the GABABR2.
Group IX, claim(s) 60-65, drawn to an antibody that binds GABABR1/R2.
Group X, claim(s) 69, drawn to method of detecting GABABR1/R2 with an antibody.
Group XI, claim(s) 72 and 76 drawn to method of identifying antagonist or antagonist of GABABR1/R2 using a transgenic.
Group XII, claim(s) 73-74, drawn to an antagonist of GABABR1/R2 and pharmaceutical composition.
Group XIII, claim(s) 75, drawn to method of treating an abnormality by decreasing GABABR1/R2 activity.
Group XIV, claim(s) 77-78, drawn to an agonist of GABABR1/R2 and pharmaceutical composition.
Group XV, claim(s) 79, drawn to method of treating an abnormality by increasing GABABR1/R2 activity.
Group XVI, claim(s) 80-81, drawn to a cell expressing GABABR1/R2.
Group XVII, claim(s) 82-91, 93-97, drawn to a method of identifying chemicals which bind to GABABR1/R2.
Group XVIII, claim(s) 92, 98, 182, 186, 189, drawn to compound which binds GABABR1/R2.
Group XIX, claim(s) 99-112, 114-119, 148-156, drawn to competitive binding assay.
Group XX, claim(s) 113, 182, 185, 186, 189, drawn to compound which binds GABABR1/R2 and successfully competes with a compound known to bind GABABR1/R2.
Group XXI, claim(s) 120-123, 128-142, 159-171, drawn to a method of detecting an agonist of receptor activity.
Group XXII, claim(s) 121-125, 143-145, 172, 183, 185, 187, 189, drawn to agonist and method of synthesis.
Group XXIII, claim(s) 126, 127, 143, 146, 147, 172, 184, 185, 188, 189, drawn to antagonist and method of synthesis.
Group XXIV, claim(s) 148, 156, drawn to method of identifying compounds which activate GABABR1/R2 by screening plurality of compounds not known to bind GABABR1/R2.
Group XXV, claim(s) 157, 158, drawn pharmaceutical composition identified by screening plurality of compounds.
Group XXVI, claim(s) 173, drawn to method of treating spasticity.
Group XXVII, claim(s) 174, drawn to method of treating asthma.
Group XXIII, claim(s) 175, drawn to method of treating incontinence.
Group XXIX, claim(s) 176, drawn to method of decreasing nocicpetion.
Group XXX, claim(s) 177, drawn to use of agonist as antitussive agent.
Group XXXI, claim(s) 178, drawn of method of treating drug addiction.
Group XXXII, claim(s) 179, drawn to method of treating Alzheimer's.

The inventions listed as Groups I-XXXII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Pursuant to 37 C.F.R. 1.475(d), this Authority considers that the main invention in the instant application comprises the first-recited product, nucleic acid encoding GABABR2, and the first-recited method of using that product, namely in the method of detecting the nucleic acid. Note that there is no method of making the nucleic acid. Further, pursuant to 37 C.F.R. 1.475(d), the ISA/US considers that the materially and functionally dissimilar product of groups II-VI, IX, XII, XIV, XVI, XIII, XX, XXII, XXIII and XXV and the additional methods of groups VII,VIII, X, XI, XIII, XV, XVII, XIX, XXI, XXIV, XXVI-XXXII do not correspond to the main invention. This Authority therefore considers that the several inventions do not share a special technical feature within the meaning of PCT Rule 13.2 and thus do not relate to a single general inventive concept within the meaning of PCT Rule 13.1.

FIGURE 2A

1	M	P	S	C	P	A	R	S	A	T	G	P	L	S	I	M	G	L	M	P	20
21	L	T	K	E	V	A	K	G	S	I	G	R	G	V	L	P	A	V	E	L	40
41	A	I	E	Q	I	R	N	E	S	L	L	R	P	Y	F	L	D	L	R	L	60
61	Y	D	T	E	C	D	N	A	K	G	L	K	A	F	Y	D	A	I	K	Y	80
81	G	P	N	H	L	M	V	F	G	G	V	C	P	S	V	T	S	I	I	A	100
101	E	S	L	Q	G	W	N	L	V	Q	L	S	F	A	A	T	T	P	V	L	120
121	A	D	K	K	K	Y	P	Y	F	F	R	T	V	P	S	D	N	A	V	N	140
141	P	A	I	L	K	L	L	K	H	Y	Q	W	K	R	V	G	T	L	T	Q	160
161	D	V	Q	R	F	S	E	V	R	N	D	L	T	G	V	L	Y	G	E	D	180
181	I	E	I	S	D	T	E	S	F	S	N	D	P	C	T	S	V	K	K	L	200
201	K	G	N	D	V	R	I	I	L	G	Q	F	D	Q	N	M	A	A	K	V	220
221	F	C	C	A	Y	E	E	N	M	Y	G	S	K	Y	Q	W	I	I	P	G	240

FIGURE 2B

241	W	Y	E	P	S	W	W	E	Q	V	H	T	E	A	N	S	S	R	C	L	260
261	R	K	N	L	L	A	A	M	E	G	Y	I	G	V	D	F	E	P	L	S	280
281	S	K	Q	I	K	T	I	S	G	K	T	P	Q	Q	Y	E	R	E	Y	N	300
301	N	K	R	S	G	V	G	P	S	K	F	H	G	Y	A	Y	D	G	I	W	320
321	V	I	A	K	T	L	Q	R	A	M	E	T	L	H	A	S	S	R	H	Q	340
341	R	I	Q	D	F	N	Y	T	D	H	T	L	G	R	I	I	L	N	A	M	360
361	N	E	T	N	F	F	G	V	T	G	Q	V	V	F	R	N	G	E	R	M	380
381	G	T	I	K	F	T	Q	F	Q	D	S	R	E	V	K	V	G	E	Y	N	400
401	A	V	A	D	T	L	E	I	I	N	D	T	I	R	F	Q	G	S	E	P	420
421	P	K	D	K	T	I	I	L	E	Q	L	R	K	I	S	L	P	L	Y	S	440
441	I	L	S	A	L	T	I	L	G	M	I	M	A	S	A	F	L	F	F	N	460
461	I	K	N	R	N	Q	K	L	I	K	M	S	S	P	Y	M	N	N	L	I	480

FIGURE 2C

481	I	L	G	G	M	L	S	Y	A	S	I	F	L	F	G	L	D	G	S	F	500
501	V	S	E	K	T	F	E	T	L	C	T	V	R	T	W	I	L	T	V	G	520
521	Y	T	T	A	F	G	A	M	F	A	K	T	W	R	V	H	A	I	F	K	540
541	N	V	K	M	K	K	K	I	I	K	D	Q	K	L	L	V	I	V	G	G	560
561	M	L	L	I	D	L	C	I	L	I	C	W	Q	A	V	D	P	L	R	R	580
581	T	V	E	K	Y	S	M	E	P	D	P	A	G	R	D	I	S	I	R	P	600
601	L	L	E	H	C	E	N	T	H	M	T	I	W	L	G	I	V	Y	A	Y	620
621	K	G	L	L	M	L	F	G	C	F	L	A	W	E	T	R	N	V	S	I	640
641	P	A	L	N	D	S	K	Y	I	G	M	S	V	Y	N	V	G	I	M	C	660
661	I	I	G	A	A	V	S	F	L	T	R	D	Q	P	N	V	Q	F	C	I	680
681	V	A	L	V	I	I	F	C	S	T	I	T	L	C	L	V	F	V	P	K	700
701	L	I	T	L	R	T	N	P	D	A	A	T	Q	N	R	R	F	Q	F	T	720

FIGURE 2D

```
721  Q N Q K K E D S K T S T S V T S V N Q A  740
741  S T S R L E G L Q S E N H R L R M K I T  760
761  E L D K D L E E V T M Q L Q D T P E K T  780
781  T Y I K Q N H Y Q E L N D I L N L G N F  800
801  T E S T D G G K A I L K N H L D Q N P Q  820
821  L Q W N T T E P S R T C K D P I E D I N  840
841  S P E H I Q R R L S L Q L P I L H H A Y  860
861  L P S I G G V D A S C V S P C V S P T A  880
881  S P R H R H V P P S F R V M V S G L      898
```

FIGURE 3A

```
1    ATGGGCCTCATGCCGCTCACCAAGGAGGTGGCCAAGGGCAGCATCGGGCGCGGCGTGCTC  60
61   CCCGCCGTGGAGCTAGCCATCGAGCAGATCCGCAACGAGTCACTCCTGCGCCCCTACTTC  120
121  CTGGACCTGCGACTCTATGACACCGAGTGTGACAATGCAAAGgGACTGAAAGCCTTCTAT  180
181  GACGCAATAAAGTATGGGCTGAACCATTTGATGGTGTTTGGAGGCGTCTGTCCGTCTGTC  240
241  ACATCTATTATCGCGGAGTCCCTCCAAGGCTGGAATCTGGTGCAGCTTTCCTTCGCCGCC  300
301  ACCACGCCTGTTCTTGCGGATAAGAAGAAGTACCCGTATTTCTTCCGGACGGTGCCGTCA  360
361  GACAACGCGGTGAACCCCGCCATCCTGAAGCTCCTGAAGCACTTCCGCTGGCGGCGTGTG  420
421  GGCACACTCACGCAGGACGTGCAGCGCTTCTCCGAGGTGAGGAATGACCTGACTGGGGTT  480
481  CTGTATGGGGAAGATATTGAGATCTCAGACACAGAGAGTTTCTCCAATGATCCCTGCACC  540
541  AGCGTCAAAAAGCTCAAGGGGAATGACGTGCGGATCATCCTTGGCCAGTTTGACCAGAAT  600
601  ATGGCAGCAAAAGTCTTCTGTTGTGCCTTCGAGGAGAGCATGTTTGGCAGCAAGTACCAG  660
661  TGGATCATCCCGGGATGGTACGAGCCTGCGTGGTGGGAGCAGGTGCATGTGGAGGCCAAT  720
721  TCCTCACGCTGCCTGCGCAGAAGCCTCCTGGCTGCCATGGAAGGTTACATCGGAGTGGAC  780
```

FIGURE 3B

```
781   TTTGAGCCCCTGAGCTCCAAACAAATCAAGACCATCTCAGGGAAGACTCCACAGCAGTAT   840
841   GAAAGAGAGTACAACAGCAAACGTTCAGGCGTGGGCCCAGCAAGTTCCATGGGTACGCC    900
901   TACGATGGGATCTGGGTCATCGCCAAGACCCTACAGAGGGCCATGGAGACACTGCATGCC   960
961   AGTAGCAGGCACCAGCGGATCCAGGACTTCAACTACACAGACCACACGCTGGGCAAAATC   1020
1021  ATCCTCAATGCCATGAACGAGACCAACTTCTTCGGGGTCACGGGTCAAGTTGTGTTCCGG   1080
1081  AACGGGGAGAGAATGGGAACCATTAAATTTACTCAATTTCAAGACAGCAGAGAGGTGAAG   1140
1141  GTCGGCGAATACAACGCGGTGGCTGACACACTGGAGATCATCAATGACACCATAAGGTTC   1200
1201  CAGGGGTCCGAGCCACCCAAGGACAAGACCATCATTCTGGAGCAGCTTCGGAAGATCTCG   1260
1261  CTTCCACTGTATAGCATCCTGTCCGCTCTCACCATCCTCGGCATGATCATGGCCAGCGCC   1320
1321  TTCCTCTTCTTCAACATCAAGAACCGGAACCAAAAGCTGATTAAGATGTCAAGCCCCTAC   1380
1381  ATGAACAACCTCATCATCCTGGGAGGAATGCTGTCCTATGCATCCATCTTCCTCTTTGGC   1440
1441  CTCGATGGGTCCTTCGTCTCAGAAAAGACCTTTGAAACACTCTGCACGGTCCGGACCTGG   1500
1501  ATTCTCACCGTGGGCTACACAACTGCCTTTGGGGCCATGTTTGCAAAGACCTGGAGGGTC   1560
```

FIGURE 3C

1561	CATGCCATCTTCAAAAATGTGAAGATGAAGAAGATCATCAAAGACCAGAAGCTGCTT	1620
1621	GTGATTGTGGGGGCATGCTGCTCATCGACCTGTGCATCCTGATCTGTTGGCAGGCTGTG	1680
1681	GACCCCCTGCGGAGGACAGTAGAGAGGTACAGCATGGAGCCGGACCCAGCAGGCCGGGAC	1740
1741	ATCTCCATCCGCCCATTGCTGGAACACTGCGAAAACACCCACATGACCATCTGGCTTGGC	1800
1801	ATTGTCTACGCCTACAAGGGGCTCCTCATGCTATTCGGTTGTTTCTTGGCATGGGAAACC	1860
1861	CGCAATGTGAGCATCCCTGCCCTCAACGACAGCAAGTACATCGGCATGAGTGTGTACAAT	1920
1921	GTGGGGATCATGTGCATCATCGGGGCTGCTGTCTCCTTCCTGACGCGTGACCAGCCCAAC	1980
1981	GTGCAGTTCTGCATCGTGGCCCTGGTCATCATCTTCTGCAGCACCATCACTCTCTGCCTG	2040
2041	GTGTTTGTGCCAAAGCTCATTACTCTGAGGACAAACCCTGACGCAGCCACTCAGAACAGG	2100
2101	CGGTTCCAGTTCACACAGAACCAGAAGAAAGAAGATTCGAAGACCTCCACTTCAGTCACC	2160
2161	AGCGTGAACCAGGCGAGCACGTCACGCCTGGAGGGACTGCAGTCAGAAAACCACCGCCTT	2220
2221	CGAATGAAGATCACAGAGCTGGACAAAGACTTGGAAGAAGTCACCATGCAGCTACAAGAC	2280
2281	ACACCAGAGAAGACCACATACATCAAACAGAATCACTACCAAGAGCTCAACGACATCCTC	2340

FIGURE 3D

```
2341  AGCTTGGGCAACTTCACAGAGAGCACAGATGGAGGAAAGGCCATTCTAAAAAATCACCTC  2400
2401  GATCAAAACCCCCAGCTCCAGTGGAACACGACAGAGCCCTCAAGAACATGCAAAGACCCC  2460
2461  ATAGAAGACATCAACTCCCCGGAGCACATCCAGCGCCGGCTGTCGCTCCAGCTCCCCATC  2520
2521  CTTCACCACGCCTACCTCCCATCCATCGGAGGCGTGGATGCCAGCTGCGTCAGCCCCTGT  2580
2581  GTCAGCCCTACCGCCAGCCCTCGCCACAGACACGTACCACCCTCCTTCCGAGTCATGGTC  2640
2641  TCGGGCCTGTAG                                                  2652
```

FIGURE 4A

```
  1  M G L M P L T K E V A K G S I G R G V L   20
 21  P A V E L A I E Q I R N E S L L R P Y F   40
 41  L D L R L Y D T E C D N A K G L K A F Y   60
 61  D A I K Y G L N H L M V F G G V C P S V   80
 81  T S I I A E S L Q G W N L V Q L S F A A  100
101  T T P V L A D K K K Y P Y F F R T V P S  120
121  D N A V N P A I L K L L K H F R W R R V  140
141  G T L T Q D V Q R F S E V R N D L T G V  160
161  L Y G E D I E I S D T E S F S N D P C T  180
181  S V K K L K G N D V R I I L G Q F D Q N  200
201  M A A K V F C C A F E E S M F G S K Y Q  220
221  W I I P G W Y E P A W W E Q V H V E A N  240
241  S S R C L R R S L L A A M E G Y I G V D  260
```

FIGURE 4B

261	F	E	P	L	S	S	K	Q	I	K	T	I	S	G	K	T	P	Q	Q	Y	280
281	E	R	E	Y	N	S	K	R	S	G	V	G	P	S	K	F	H	G	Y	A	300
301	Y	D	G	I	W	V	I	A	K	T	L	Q	R	A	M	E	T	L	H	A	320
321	S	S	R	H	Q	R	I	Q	D	F	N	Y	T	D	H	T	L	G	K	I	340
341	I	L	N	A	M	N	E	T	N	F	F	G	V	T	G	Q	V	V	F	R	360
361	N	G	E	R	M	G	T	I	K	F	T	Q	F	Q	D	S	R	E	V	K	380
381	V	G	E	Y	N	A	V	A	D	T	L	E	I	I	N	D	T	I	R	F	400
401	Q	G	S	E	P	P	K	D	K	T	I	I	L	E	Q	L	R	K	I	S	420
421	L	P	L	Y	S	I	L	S	A	L	T	I	L	G	M	I	M	A	S	A	440
441	F	L	F	F	N	I	K	N	R	N	Q	K	L	I	K	M	S	S	P	Y	460
461	M	N	N	L	I	I	L	G	G	M	L	S	Y	A	S	I	F	L	F	G	480
481	L	D	G	S	F	V	S	E	K	T	F	E	T	L	C	T	V	R	T	W	500
501	I	L	T	V	G	Y	T	T	A	F	G	A	M	F	A	K	T	W	R	V	520

FIGURE 4C

```
521  H A I F K N V K M K K K I I K D Q K L L  540
541  V I V G G M L L I D L C I L I C W Q A V  560
561  D P L R R T V E R Y S M E P D P A G R D  580
581  I S I R P L L E H C E N T H M T I W L G  600
601  I V Y A Y K G L L M L F G C F L A W E T  620
621  R N V S I P A L N D S K Y I G M S V Y N  640
641  V G I M C I I G A A V S F L T R D Q P N  660
661  V Q F C I V A L V I I F C S T I T L C L  680
681  V F V P K L I T L R T N P D A A T Q N R  700
701  R F Q F T Q N Q K K E D S K T S T S V T  720
721  S V N Q A S T S R L E G L Q S E N H R L  740
741  R M K I T E L D K D L E E V T M Q L Q D  760
761  T P E K T T Y I K Q N H Y Q E L N D I L  780
```

FIGURE 4D

781	S L G N F T E S T D G G K A I L K N H L	800
801	D Q N P Q L Q W N T T E P S R T C K D P	820
821	I E D I N S P E H I Q R R L S L Q L P I	840
841	L H H A Y L P S I G G V D A S C V S P C	860
861	V S P T A S P R H R H V P P S F R V M V	880
881	S G L	883

FIGURE 5A

1	M	P	S	C	P	A	R	S	A	T	G	P	L	S	I	M	G	L	M	P	20
21	L	T	K	E	V	A	K	G	S	I	G	R	G	V	L	P	A	V	E	L	40
41	A	I	E	Q	I	R	N	E	S	L	L	R	P	Y	F	L	D	L	R	L	60
61	Y	D	T	E	C	D	N	A	K	G	L	K	A	F	Y	D	A	I	K	Y	80
81	G	P	N	H	L	M	V	F	G	G	V	C	P	S	V	T	S	I	I	A	100
101	E	S	L	Q	G	W	N	L	V	Q	L	S	F	A	A	T	T	P	V	L	120
121	A	D	K	K	K	Y	P	Y	F	F	R	T	V	P	S	D	N	A	V	N	140
141	P	A	I	L	K	L	L	K	H	Y	Q	W	K	R	V	G	T	L	T	Q	160
161	D	V	Q	R	F	S	E	V	R	N	D	L	T	G	V	L	Y	G	E	D	180
181	I	E	I	S	D	T	E	S	F	S	N	D	P	C	T	S	V	K	K	L	200
201	K	G	N	D	V	R	I	I	L	G	Q	F	D	Q	N	M	A	A	K	V	220
221	F	C	C	A	Y	E	E	N	M	Y	G	S	K	Y	Q	W	I	I	P	G	240
241	W	Y	E	P	S	W	W	E	Q	V	H	T	E	A	N	S	S	R	C	L	260
261	R	K	N	L	L	A	A	M	E	G	Y	I	G	V	D	F	E	P	L	S	280
281	S	K	Q	I	K	T	I	S	G	K	T	P	Q	Q	Y	E	R	E	Y	N	300
301	N	K	R	S	G	V	G	P	S	K	F	H	G	Y	A	Y	D	G	I	W	320

FIGURE 5B

```
321  V I A K T L Q R A M E T L H A S S R H Q  340
341  R I Q D F N Y T D H T L G R I I L N A M  360
361  N E T N F F G V T G Q V V F R N G E R M  380
381  G T I K F T Q F Q D S R E V K V G E Y N  400
401  A V A D T L E I I N D T I R F Q G S E P  420

421  P K D K T I I L E Q L R K I S L P L Y S  440
               I
441  I L S A L T I L G M I M A S A F L F F N  460

461  I K N R N Q K L I K M S S P Y M N N L I  480
               II
481  I L G G M L S Y A S I F L F G L D G S F  500
```

FIGURE 5C

III IV V

501 V S E K T F E T L C T V R T W I L T V G 520

521 Y T T A F G A M F A K T W R V H A I F K 540

541 N V K M K K K I I K D Q K L L V I V G G 560

561 M L L I D L C I L I C W Q A V D P L R R 580

581 T V E K Y S M E P D P A G R D I S I R P 600

601 L L E H C E N T H M T I W L G I V Y A Y 620

621 K G L L M L F G C F L A W E T R N V S I 640

FIGURE 5D

```
                               VI
641  P A L N D S K Y I G M S V Y N V G I M C  660
661  I I G A A V S F L T R D Q P N V Q F C I  680
                               VII
681  V A L V I I F C S T I T L C L V F V P K  700
701  L I T L R T N P D A A T Q N R R F Q F T  720
721  Q N Q K K E D S K T S T S V T S V N Q A  740
741  S T S R L E G L Q S E N H R L R M K I T  760
761  E L D K D L E E V T M Q L Q D T P E K T  780
781  T Y I K Q N H Y Q E L N D I L N L G N F  800
801  T E S T D G G K A I L K N H L D Q N P Q  820
821  L Q W N T T E P S R T C K D P I E D I N  840
841  S P E H I Q R R L S L Q L P I L H H A Y  860
861  L P S I G G V D A S C V S P C V S P T A  880
881  S P R H R H V P P S F R V M V S G L      898
```

Figure 6A

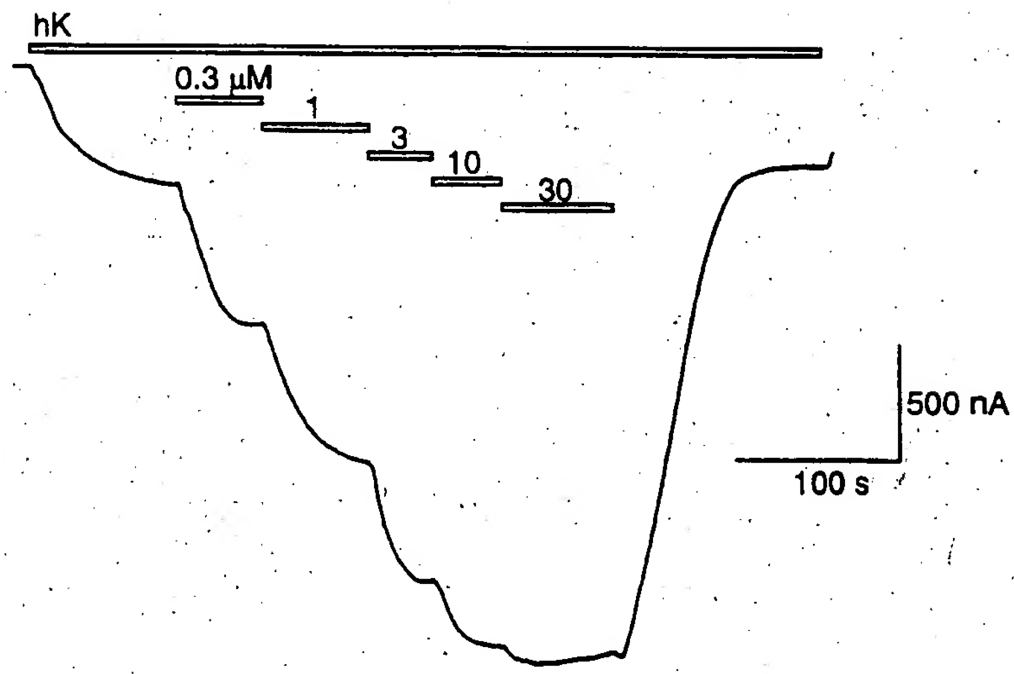


Figure 6B

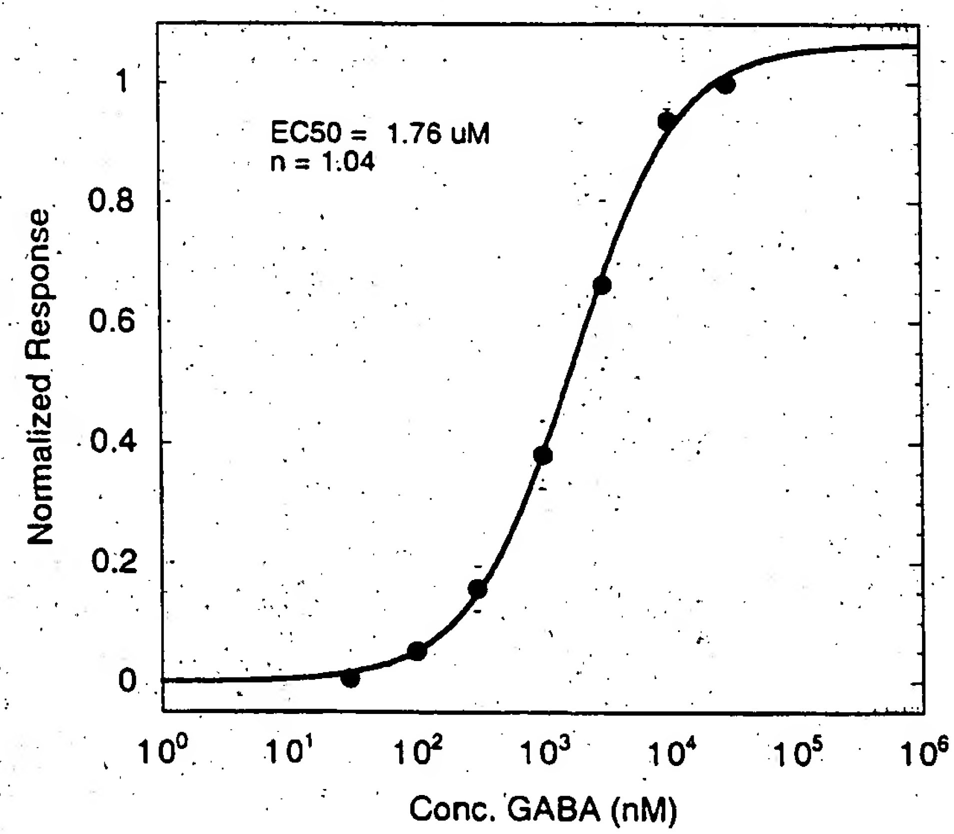


Figure 7

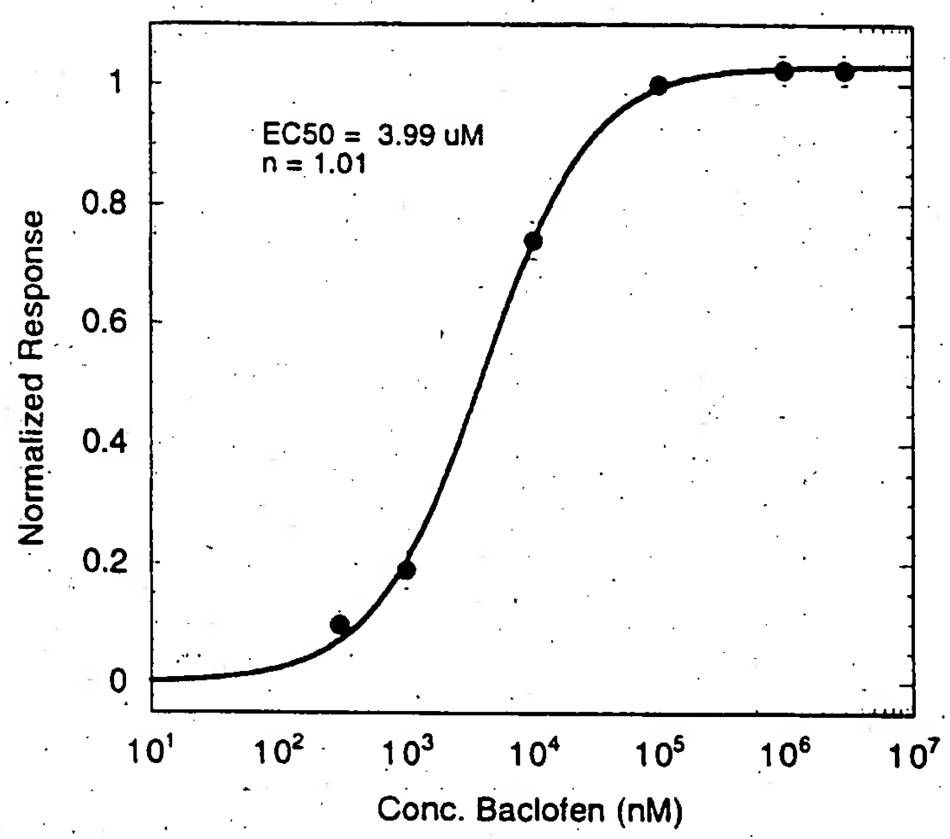

EC50 = 3.99 uM
n = 1.01
Normalized Response
0
0.2
0.4
0.6
0.8
1
10^1
10^2
10^3
10^4
10^5
10^6
10^7
Conc. Baclofen (nM)

Figure 8

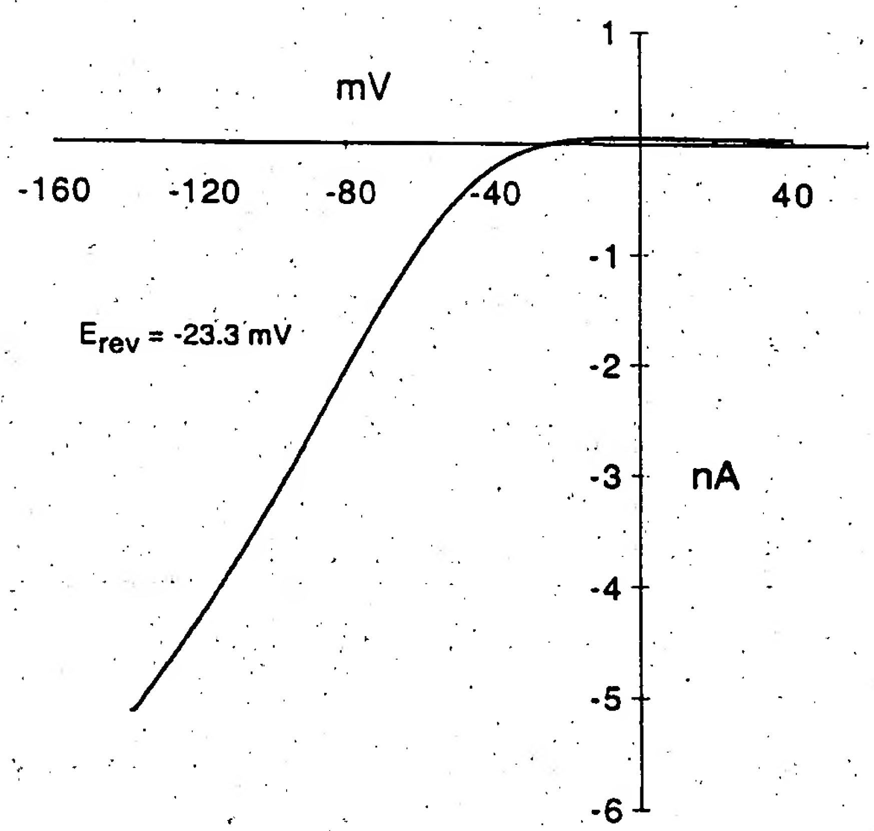


Figure 9A

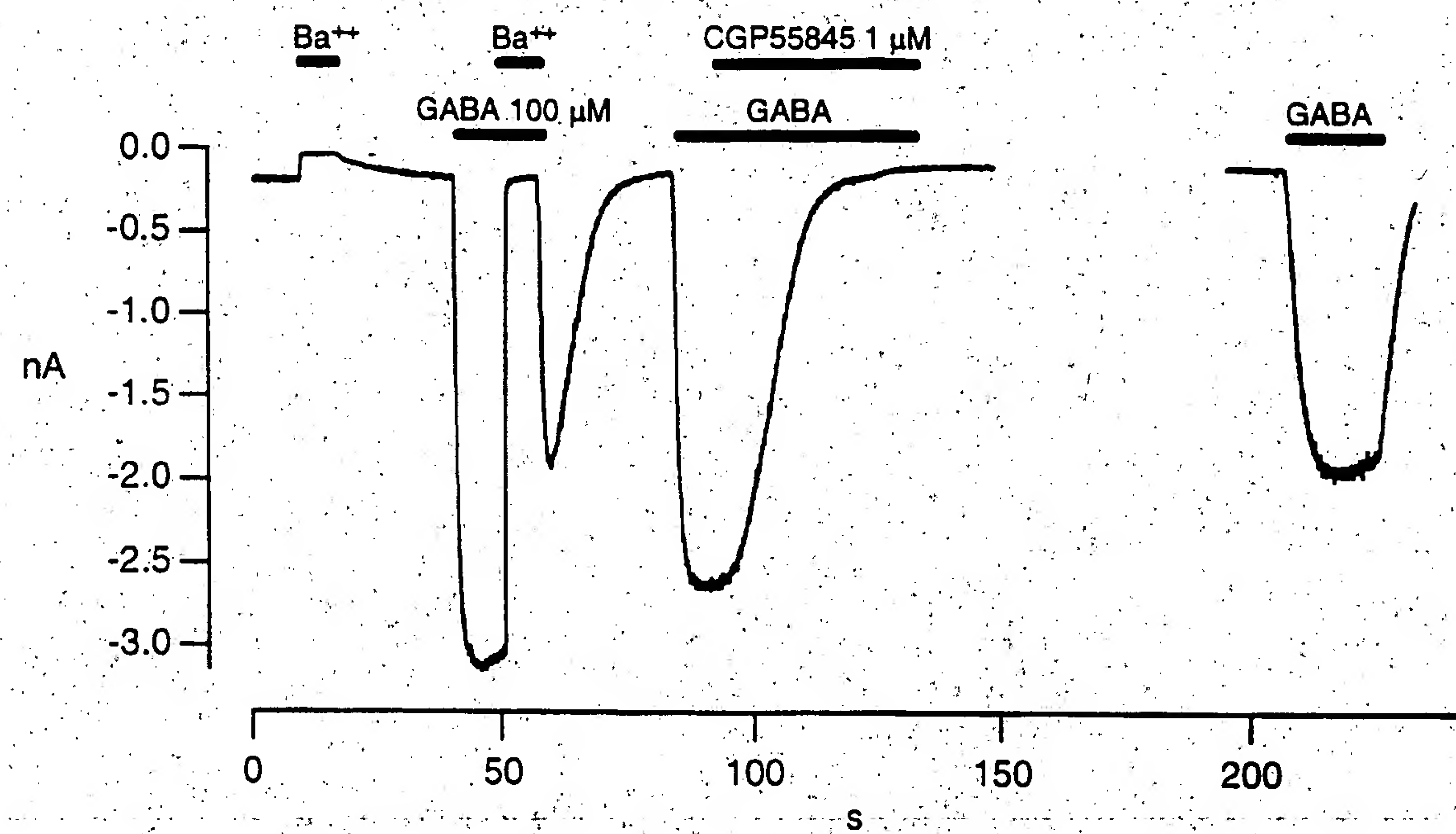


Figure 9B

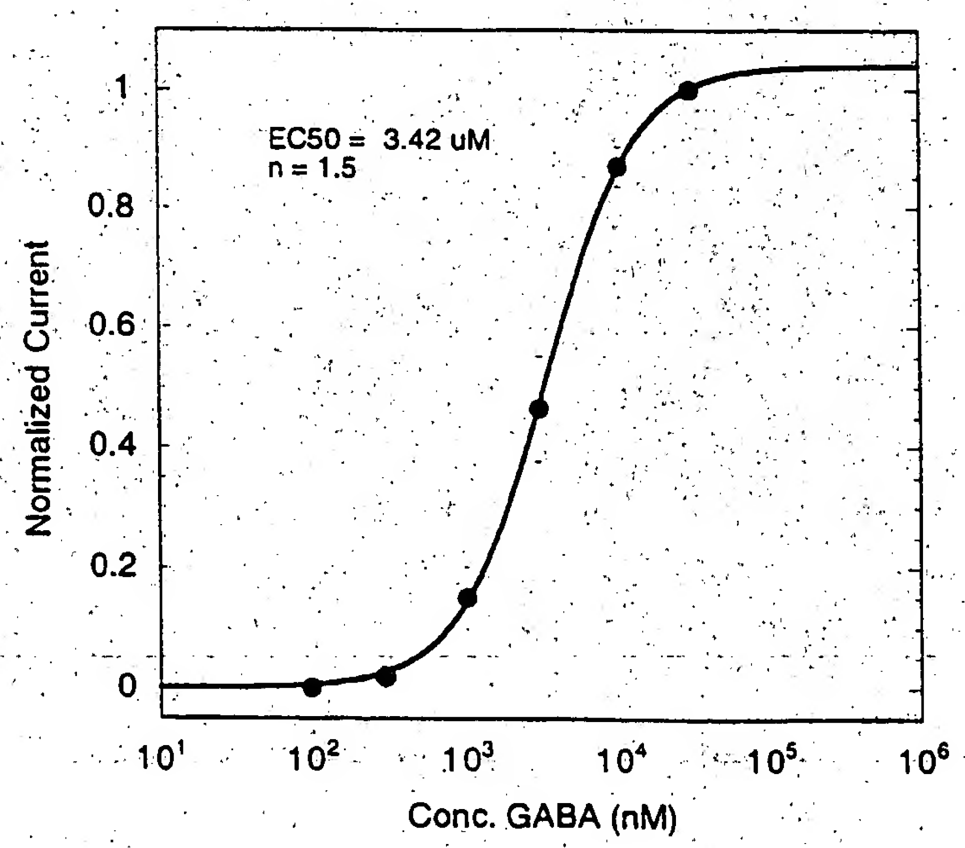


Figure 10

GABA$_B$R2	MGLMPLTKEVAKGSIGRG	18
GABA$_B$R1b	MGPGGPCTPVGWPLPLLLVMAAGVAPVWASHSPHLPRPHPRVPPHPSSERRAVYIGALFPMSGGWPGGQA	70
GABA$_B$R2	VLPAVELAIEQIRN.ESLLRPYFLDLRLYDTECDNAKGLKAFYDAIKYGLNHLMVFGGVCPSVTSIIAES	87
GABA$_B$R1b	CQPAVEMALEDVNSRRDILPDYELKLIHHDSKCDPGQATKYLYELLYNDPIKIILMPG.CSSVSTLVAEA	139
GABA$_B$R2	LQGWNLVQLSFAATTPVLADKKKYPYFFRTVPSDNAVNPAILKLLKHFRWRRVGTLTQDVQRFSEVRNDL	157
GABA$_B$R1b	ARMWNLIVLSYGSSSPALSNRQRFPTFFRTHPSATLHNPTRVKLFEKWGWKKIATIQQTTEVFTSTLDDL	209
GABA$_B$R2	TGVLYGEDIEISDTESFSNDPCTSVKKLKGNDVRIILGQFDQNMAAKVFCCAFEESMFGSKYQWIIPGWY	227
GABA$_B$R1b	EERVKEAGIEITFRQSFFSDPAVPVKNLKRQDARIIVGLFYETEARKVFCEVYKERLFGKKYVWFLIGWY	279
GABA$_B$R2	EPAWWEQVHVEANSSRCLRRSLLAAMEGYIGVDFEPLSSKQIKTISGKTPQQYEREYNSKRSGVGPSKFH	297
GABA$_B$R1b	ADNWFKTYDPSIN...CTVEEMTEAVEGHITTEIVMLNPANTRSISNMTSQEFV.EKLTKRLKRHPEETG	345
GABA$_B$R2	GY.....AYDGIWVIAKTLQRAMETLHASSRHQRIQDFNYTDHTLGKIILNAMNETNFFGVTGQVVF.RN	361
GABA$_B$R1b	GFQEAPLAYDAIWALALALNKTSGGGGRSG..VRLEDFNYNNQTITDQIYRAMNSSSFEGVSGHVVFDAS	413
GABA$_B$R2	GERMGTIKFTQFQDSREVKVGEYNAVADTLEIINDTIRFQGSEPPKDKTIILEQLRKISLPLYSILSALT	431
GABA$_B$R1b	GSRMAWTLIEQLQGGSYKKIGYYDSTKDDLS.WSKTDKWIGGSPPADQTLVIKTFRFLSQKLFISVSVLS	482
GABA$_B$R2	ILGMIMASAFLFFNIKNRNQKLIKMSSPYMNNLIILGGMLSYASIFLFGLDGSFVSEKTFETLCTVRTWI	501
GABA$_B$R1b	SLGIVLAVVCLSFNIYNSHVRYIQNSQPNLNNLTAVGCSLALAAVFPLGLDGYHIGRSQFPFVCQARLWL	552
GABA$_B$R2	LTVGYTTAFGAMFAKTWRVHAIFKNVKMKK...KIIKDQKLLVIVGGMLLIDLCILICWQAVDPLRRTVE	568
GABA$_B$R1b	LGLGFSLGYGSMFTKIWWVHTVFTKKEEKKEWRKTLEPWKLYATVGLLVGMDVLTLAIWQIVDPLHRTIE	622
GABA$_B$R2	RYSMEPDPAGRDISIRPLLEHCENTHMTIWLGIVYAYKGLLMLFGCFLAWETRNVSIPALNDSKYIGMSV	638
GABA$_B$R1b	TFAKEEPKEDIDVSILPQLEHCSSKKMNTWLGIFYGYKGLLLLLGIFLAYETKSVSTEKINDHRAVGMAI	692
GABA$_B$R2	YNVGIMCIIGAAVSFLTRDQPNVQFCIVALVIIFCSTITLCLVFVPKLITLRTNPDAATQNRRFQFTQNQ	708
GABA$_B$R1b	YNVAVLCLITAPVTMILSSQQDAAFAFASLAIVFSSYITLVVLFVPKMRRLITRGE.........WQSE	752
GABA$_B$R2	KKEDSKTSTSVTSVNQASTSRLEGLQSENHRLRMKITELDKDLEEVTMQLQDTPEKTTYIKQNHYQELND	778
GABA$_B$R1b	TQDTMKTGSS.TNNNEEEKSRL..LEKENRELEKIIAEKEERVSELRHQLQSRQQLRSRRHPPTPPDPSG	819
GABA$_B$R2	ILSLGNFTESTDGGKAILKNHLDQNPQLQWNTTEPSRTCKDPIEDINSPEHIQRRLSLQLPILHHAYLPS	848
GABA$_B$R1b	GLPRGPSEPPDRLSCDGSRVHLLYK*	844
GABA$_B$R2	IGGVDASCVSPCVSPTASPRHRHVPPSFRVMVSGL*	883

FIG. 11A

FIG. 11B

FIG. 11E

FIG. 11D

FIG. 12A

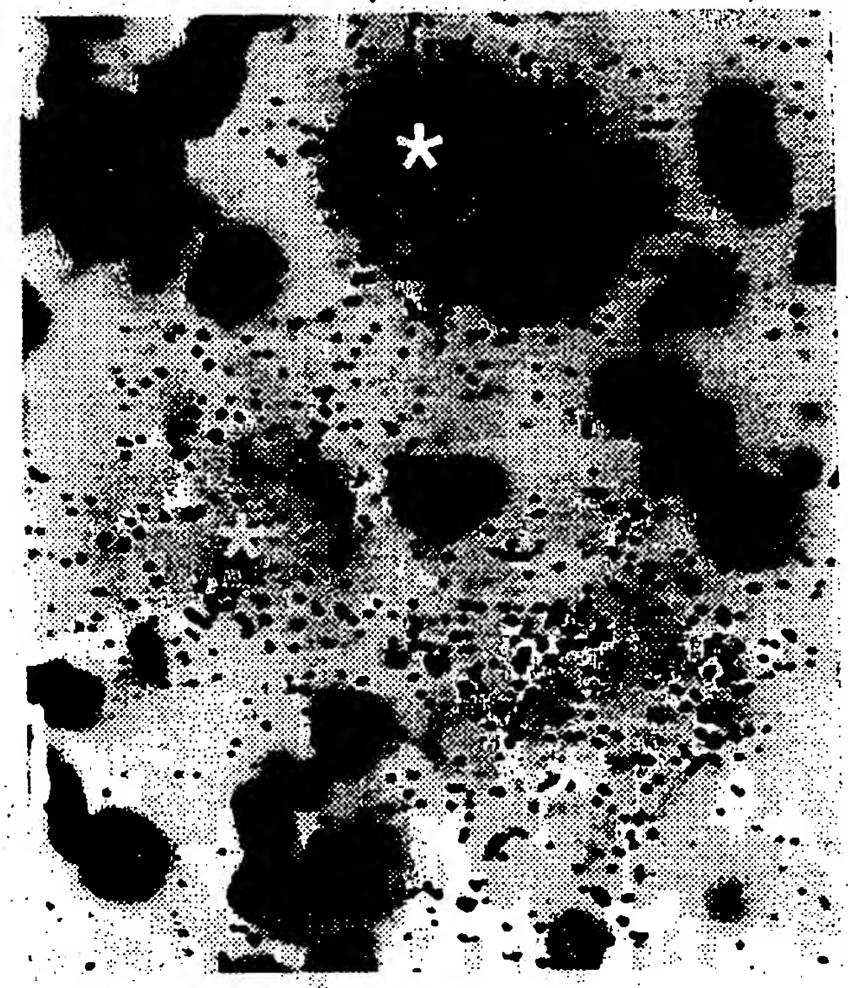


FIG. 12B

Figure 13A

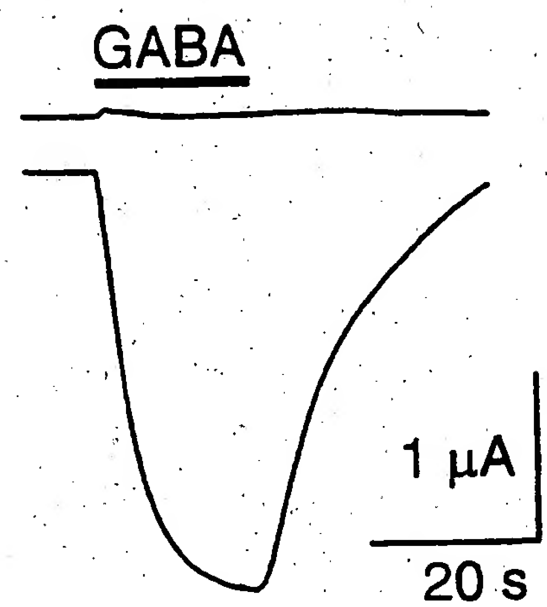


Figure 13B

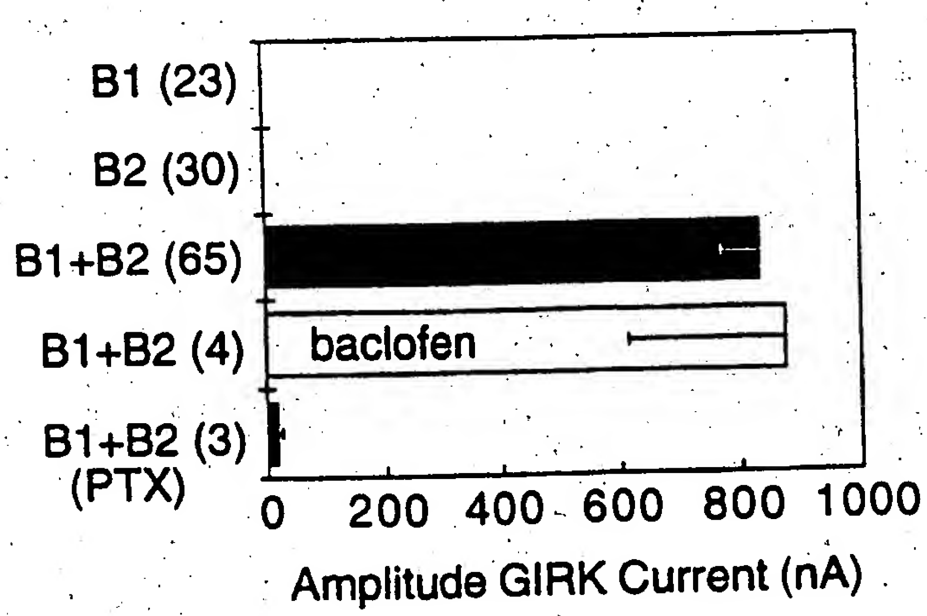


Figure 14A

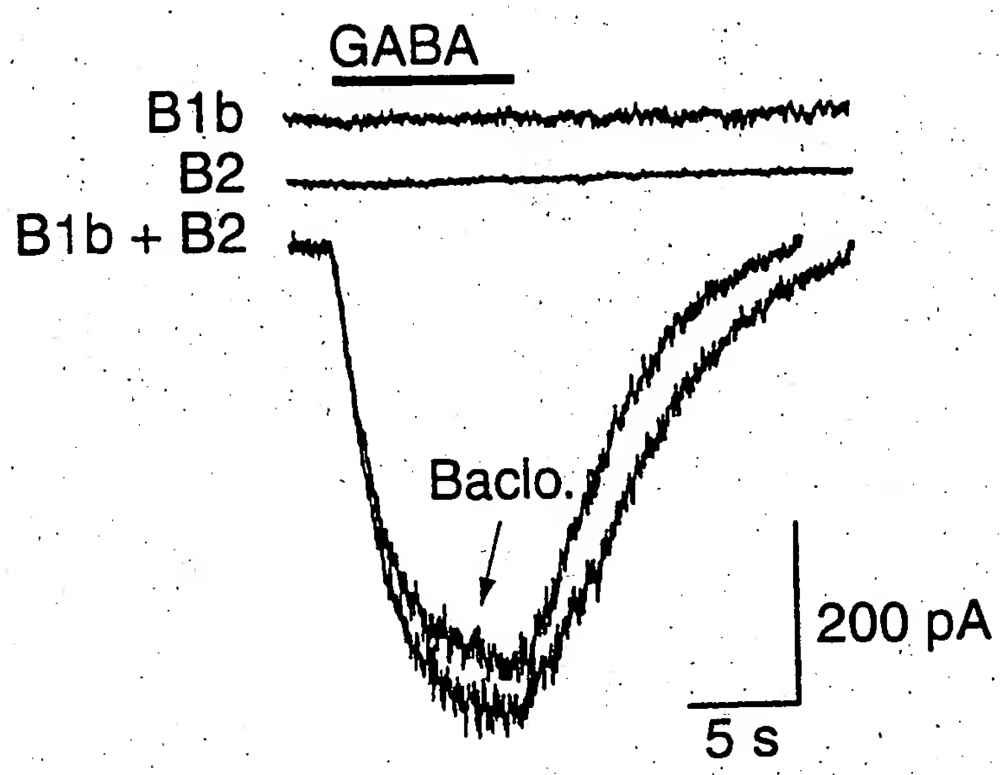


Figure 14B

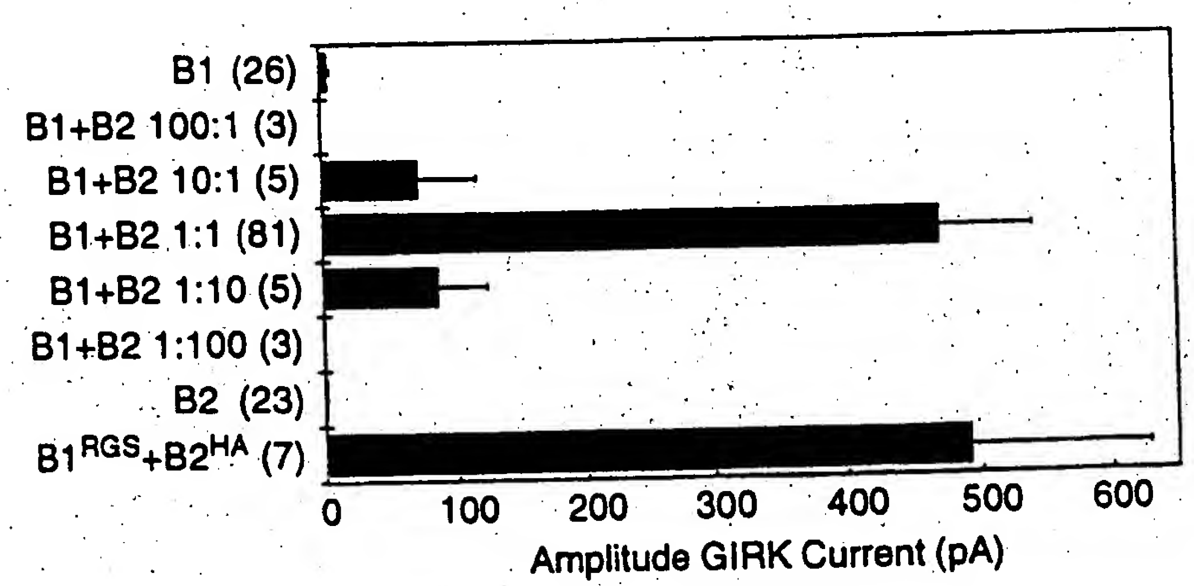


Figure 15A

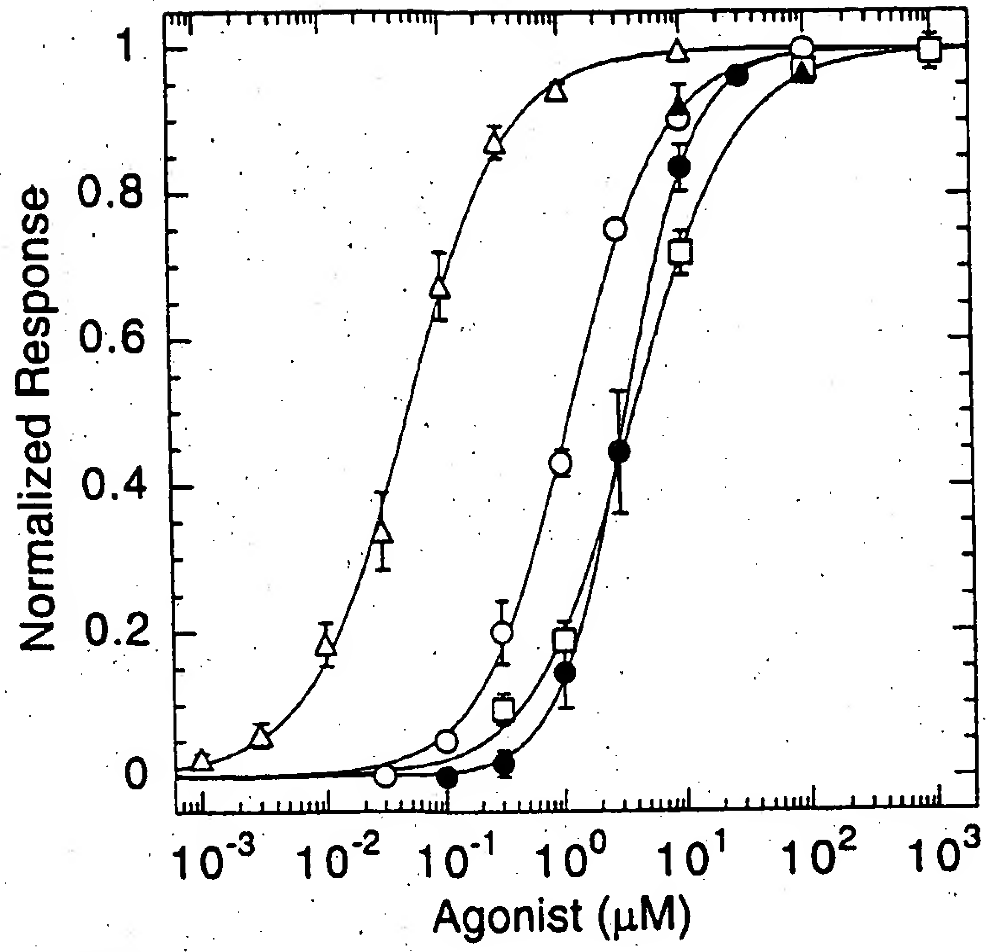


Figure 15B

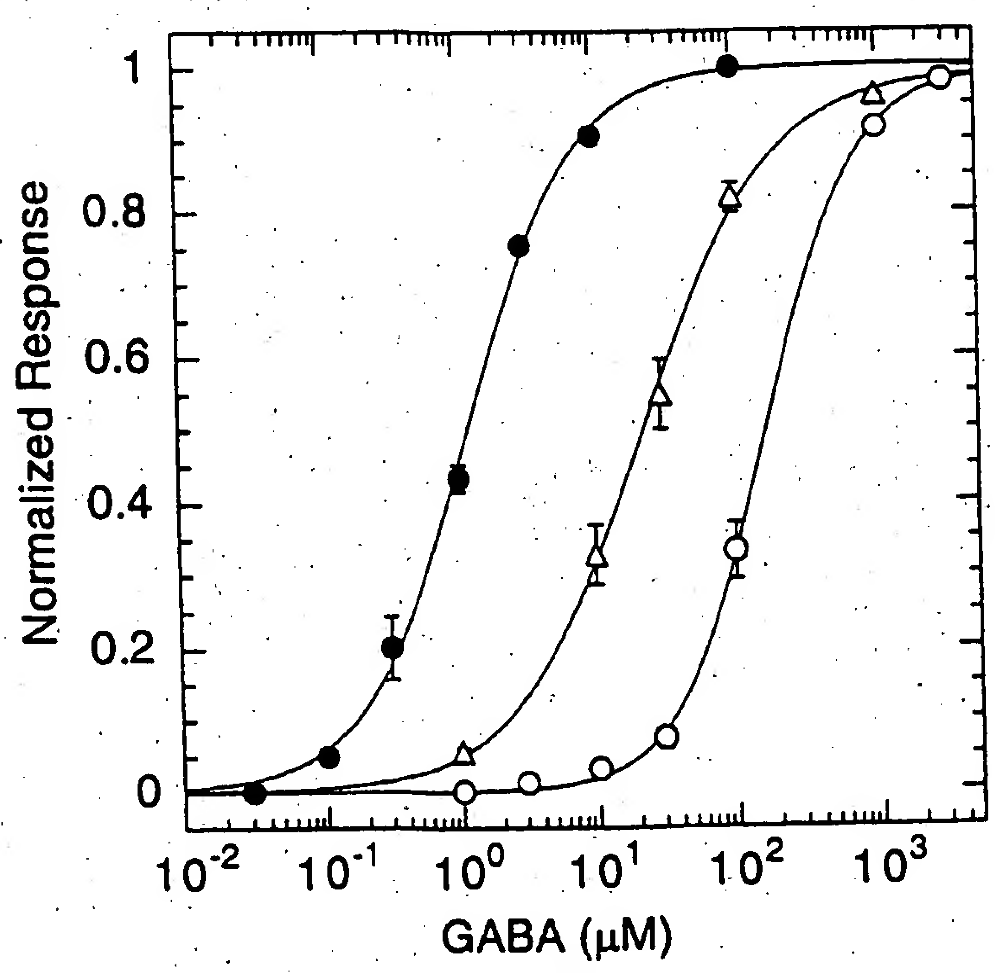


Figure 16

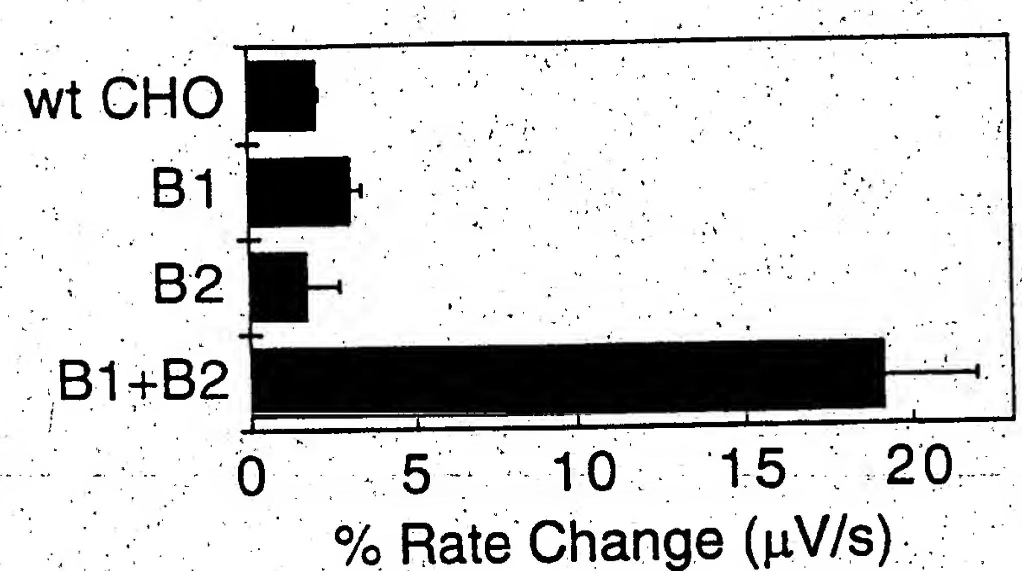


FIG. 17A

FIG. 17B

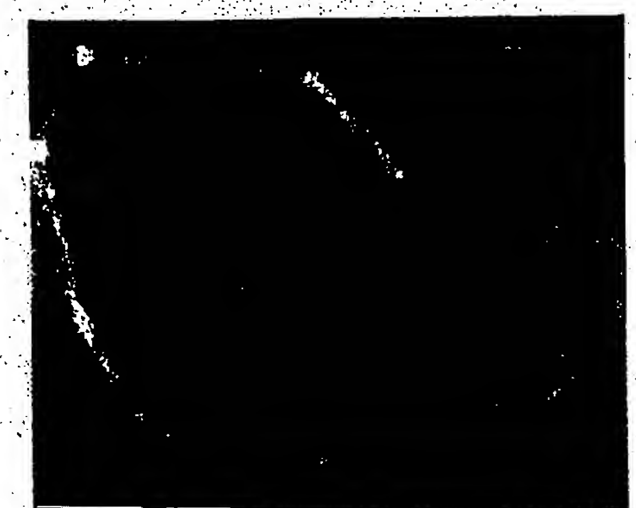


FIG. 17C

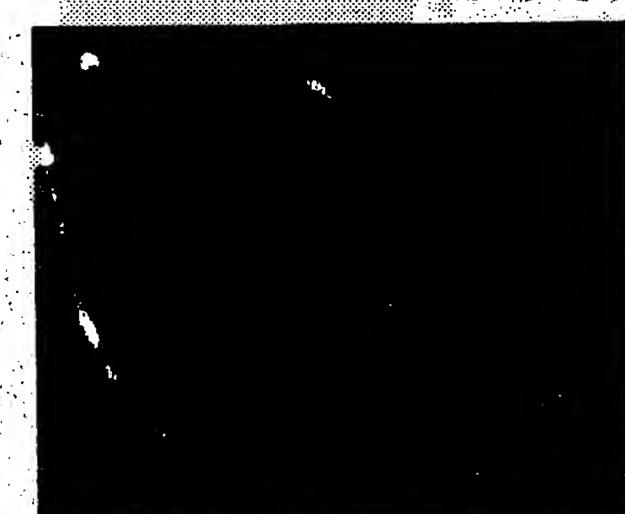


FIG. 17D

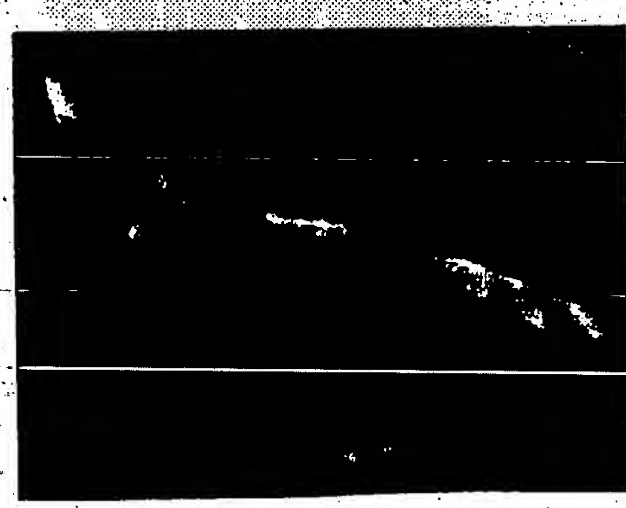


FIG. 18A

FIG. 18B

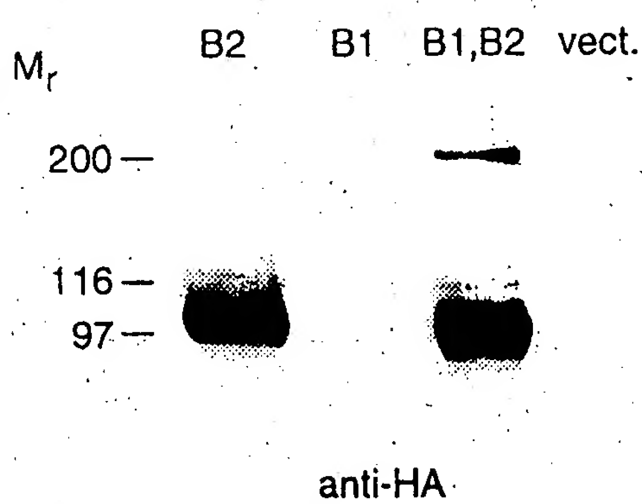


FIG. 18C

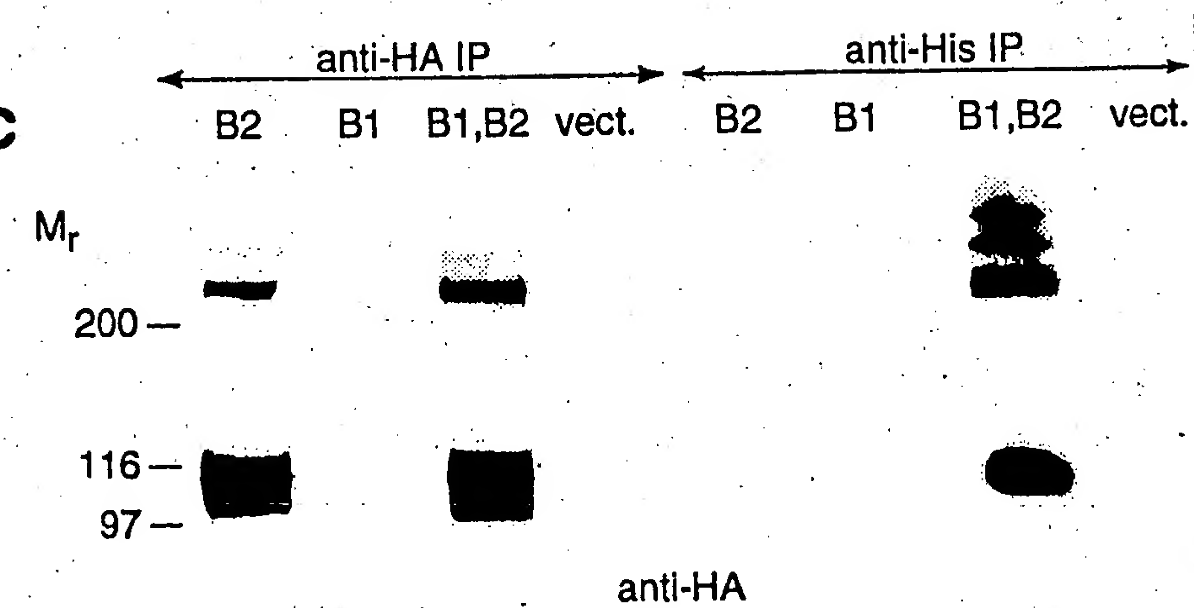


FIG. 19A

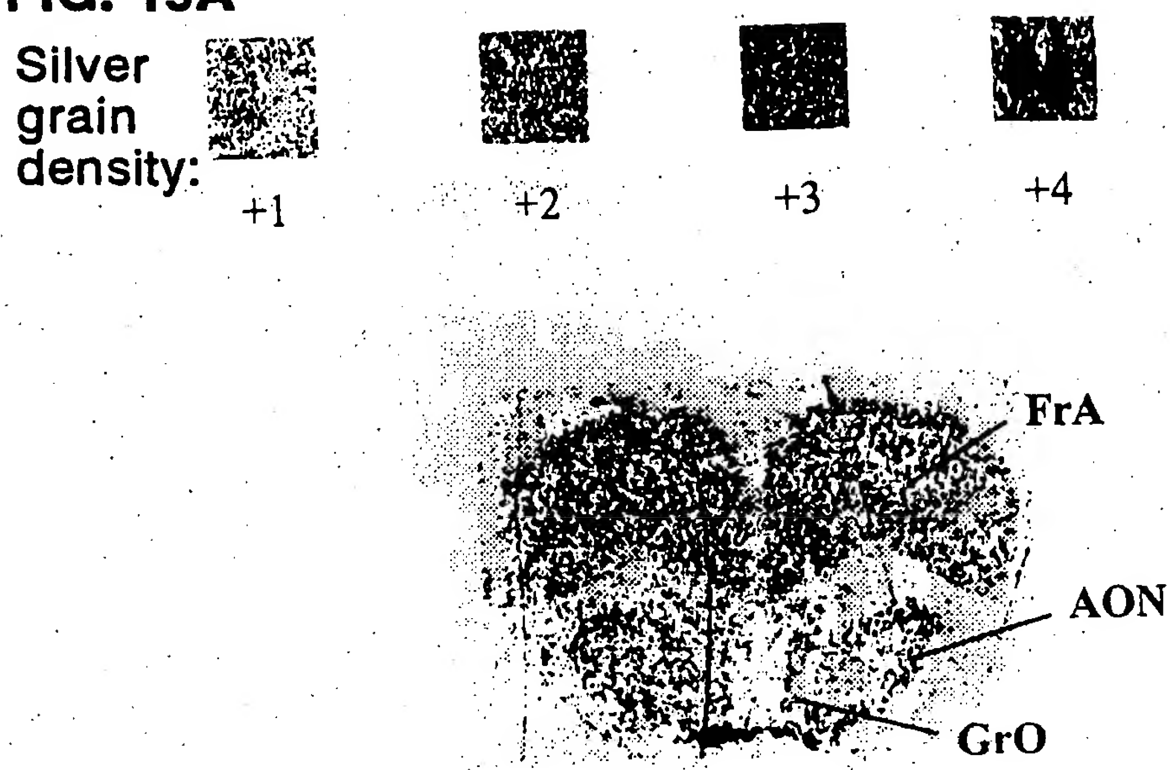


FIG. 19B

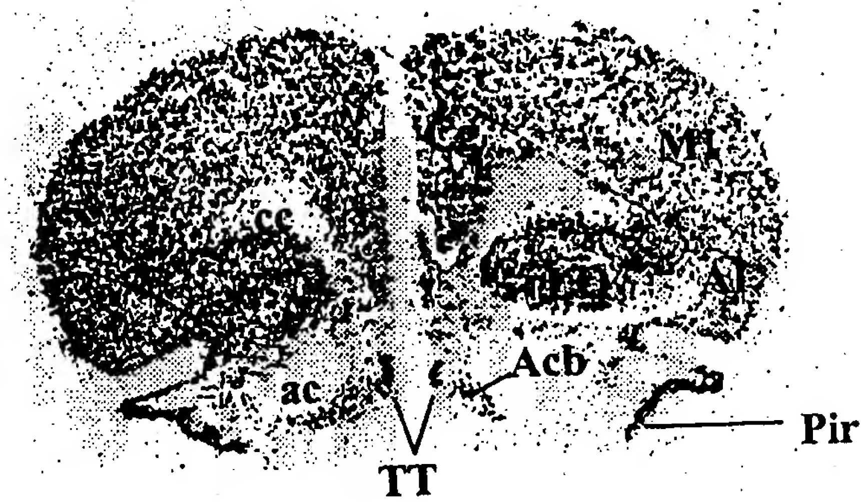


FIG. 19C

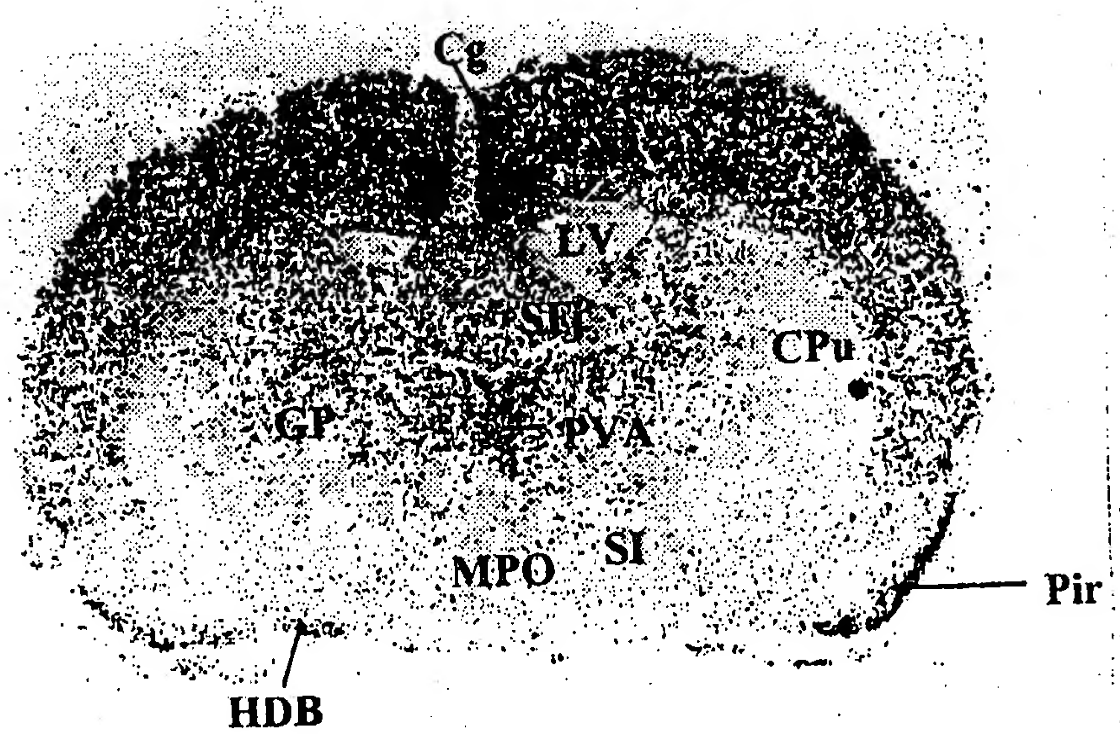


FIG. 19D

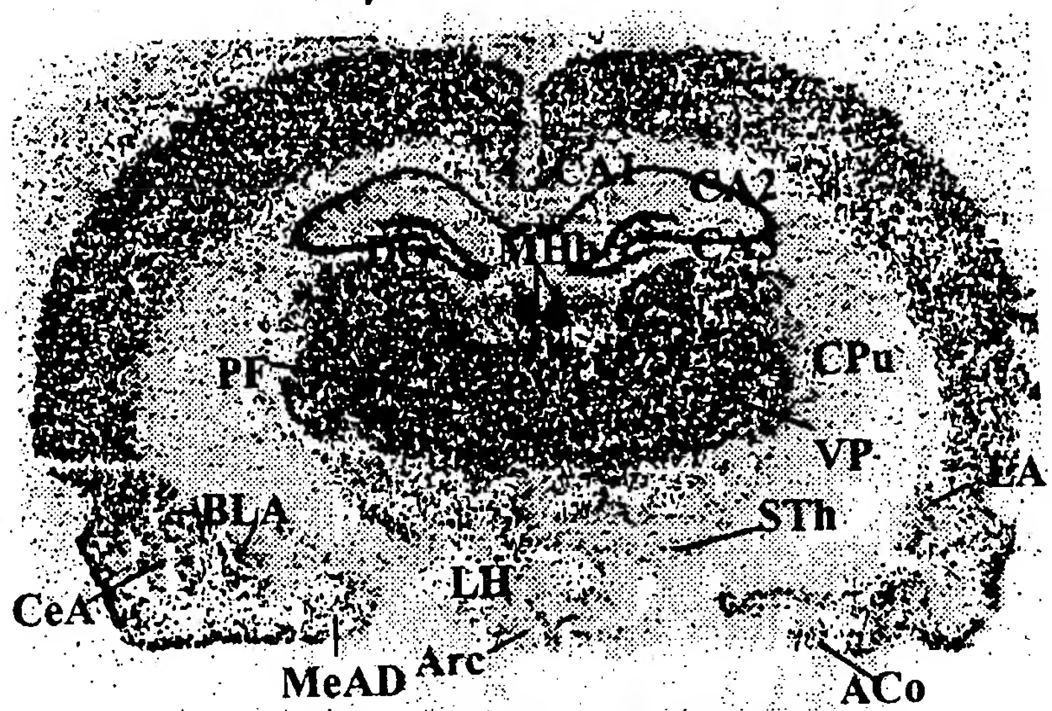


FIG. 19E

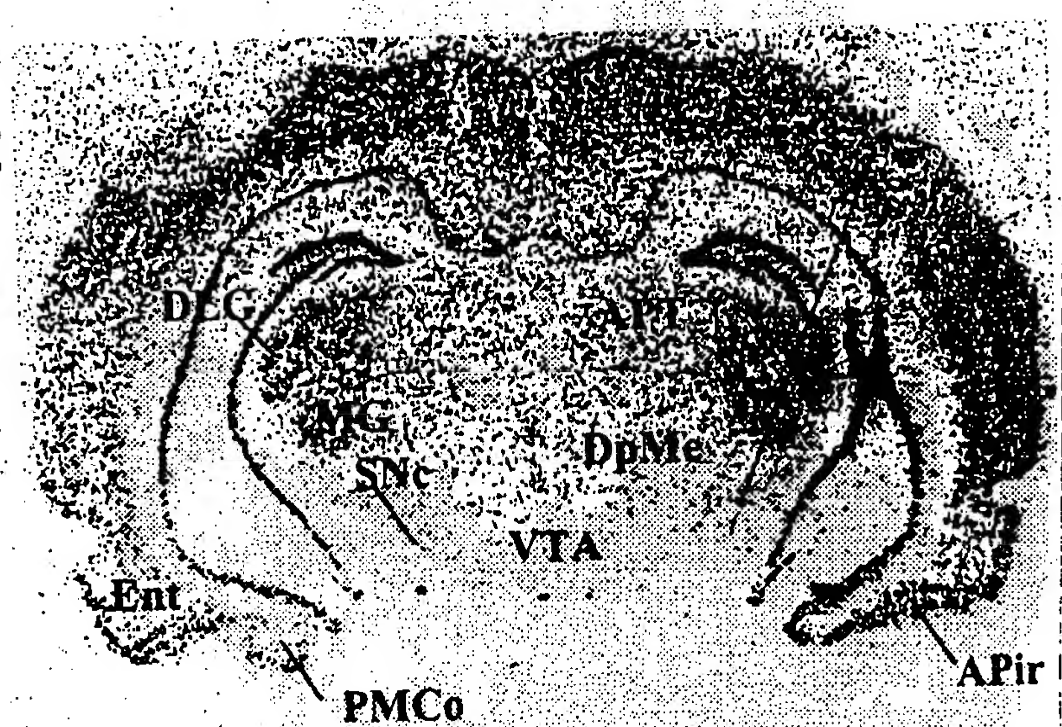


FIG. 19F

FIG. 19G

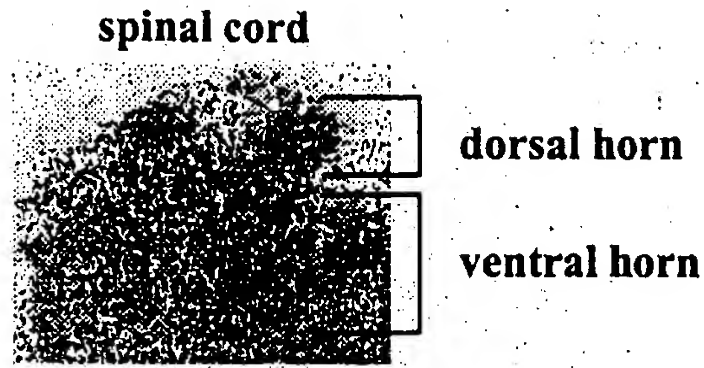


FIG. 19H

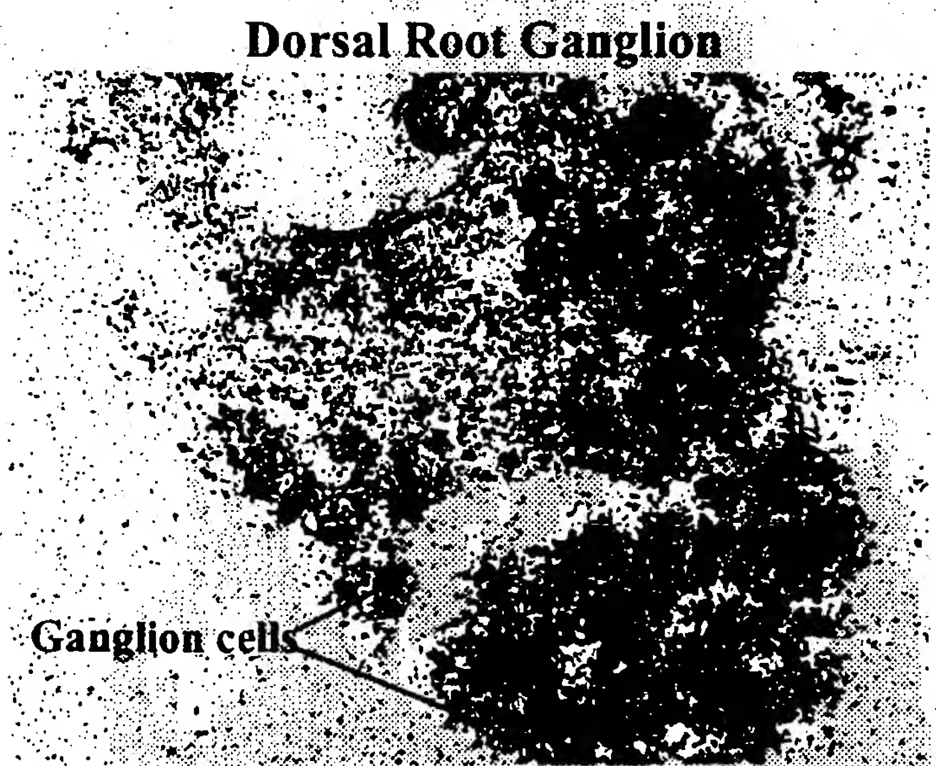


FIG. 19I

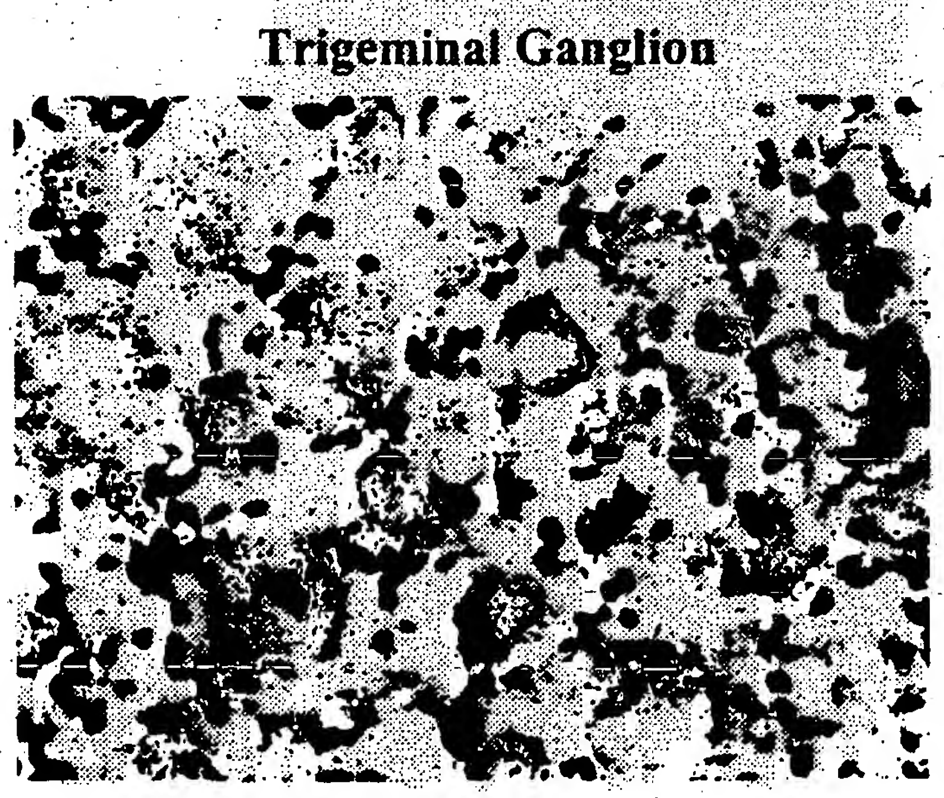


FIG. 20A

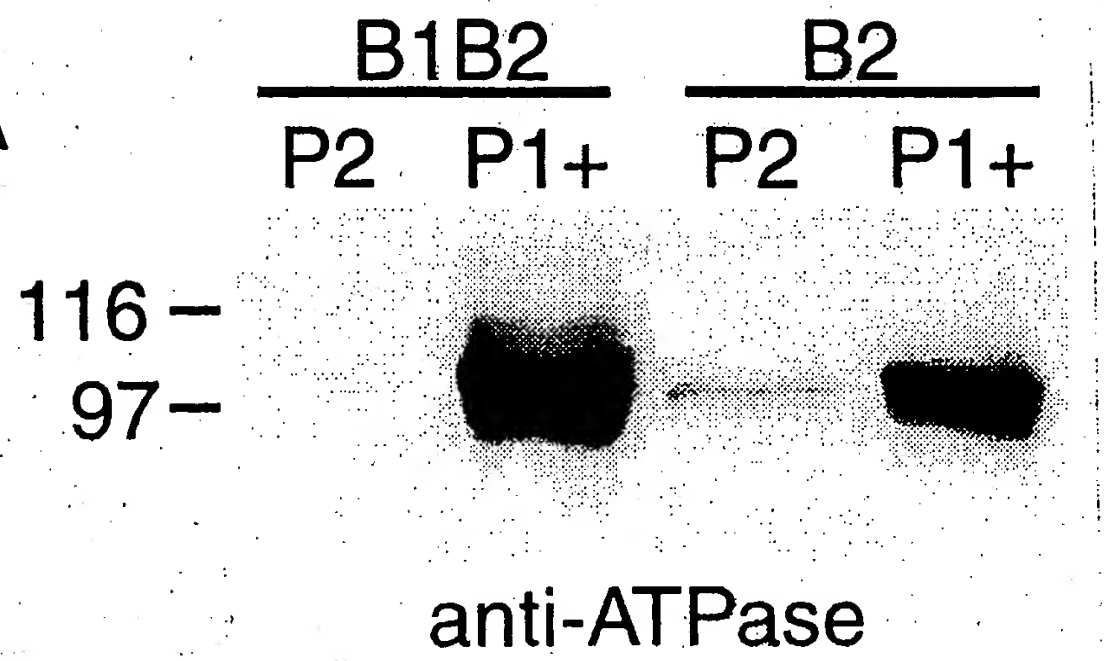


FIG. 20B

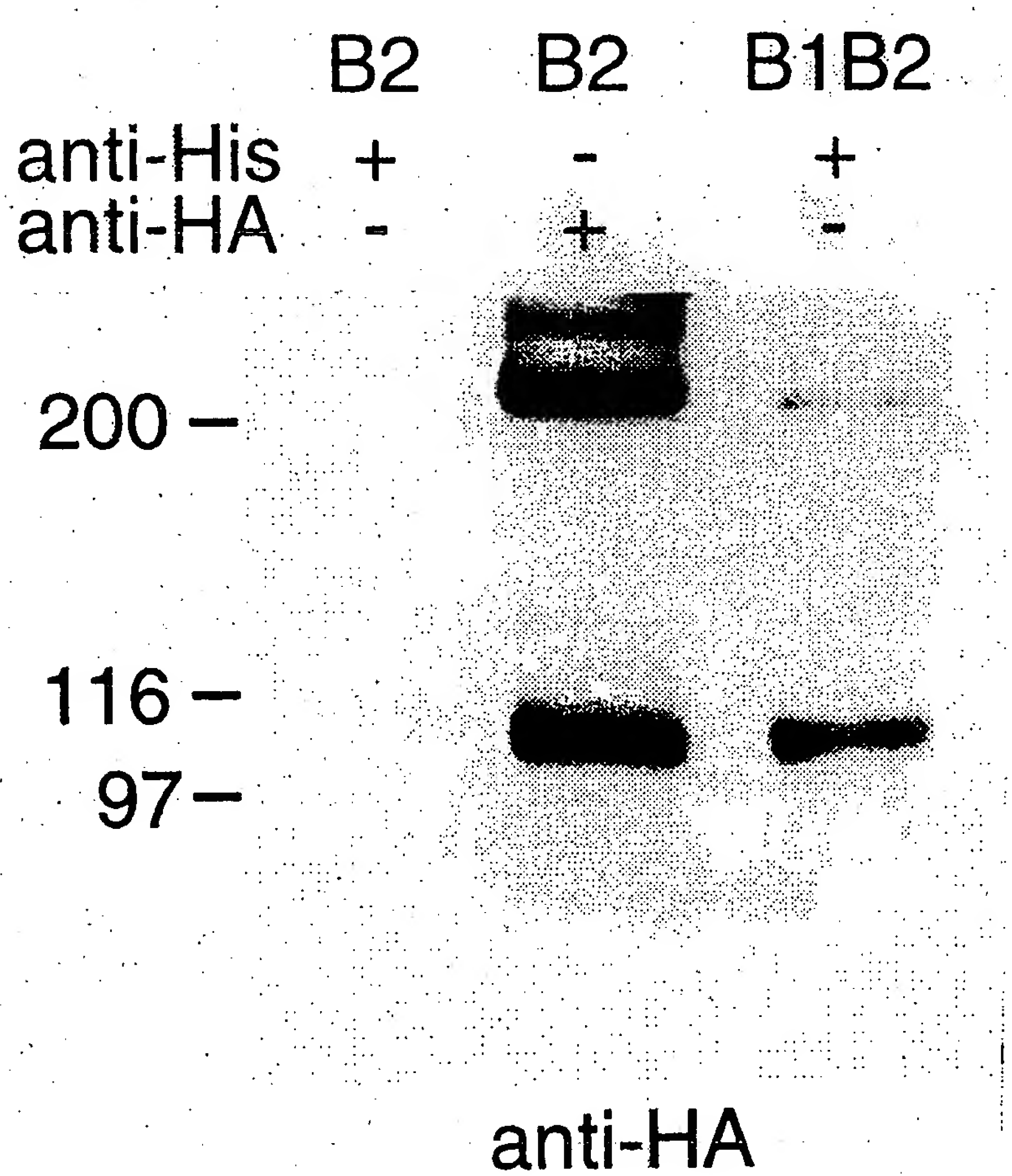


FIG. 20C

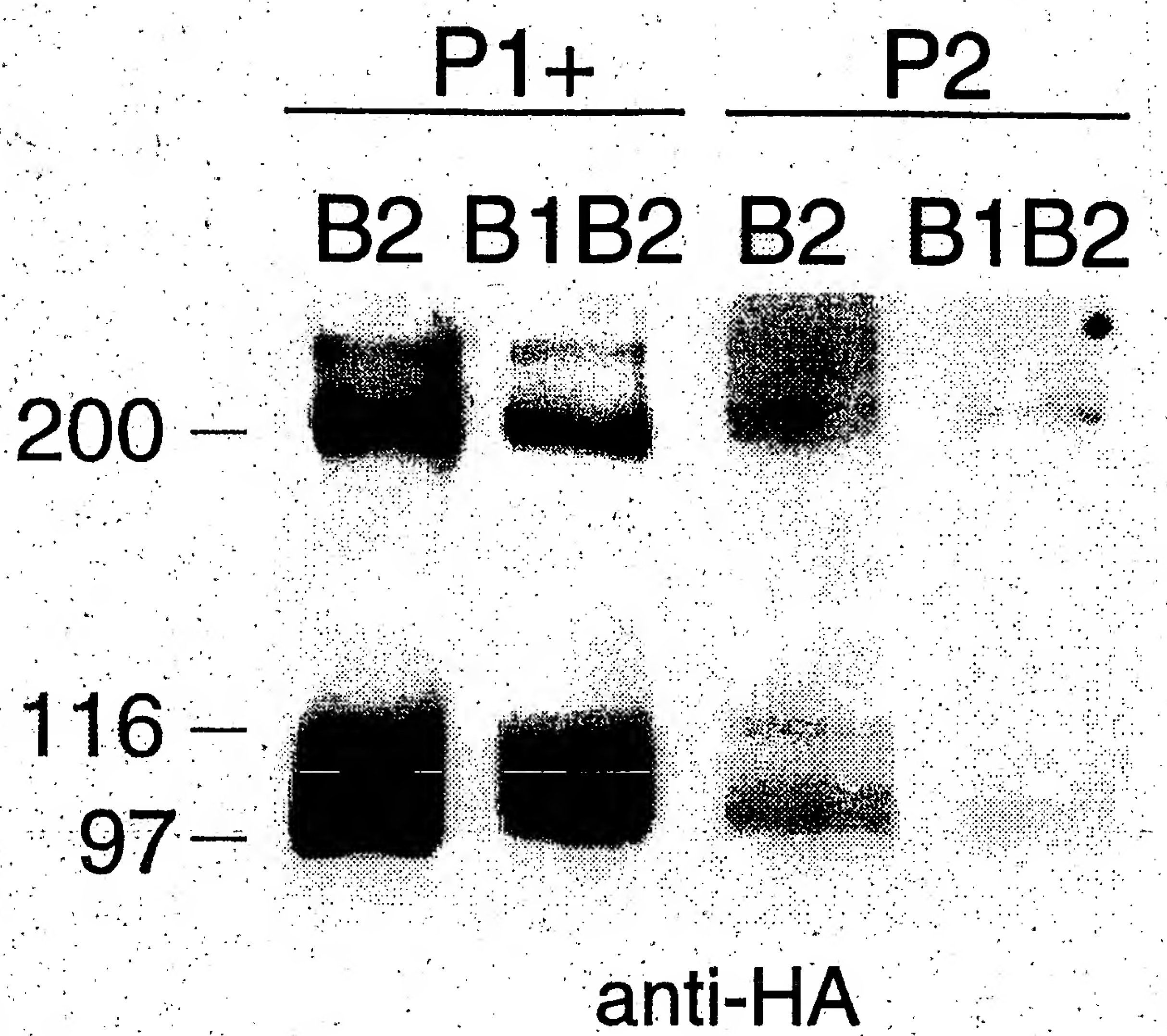


FIG. 21A

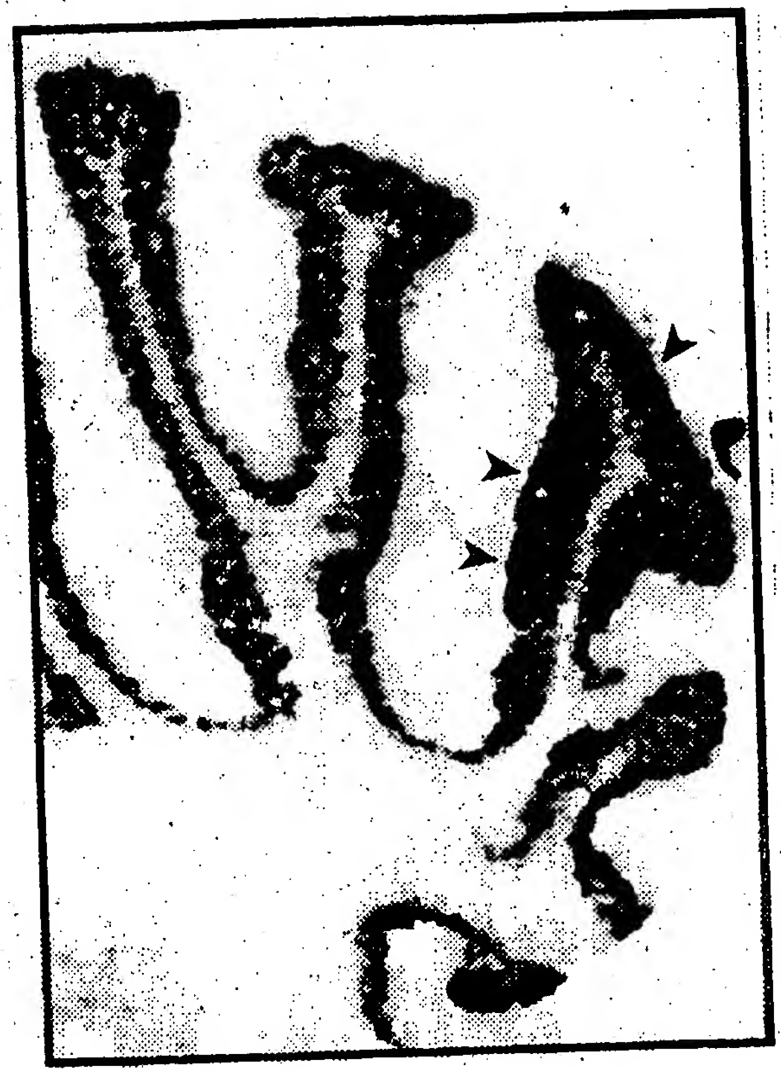


FIG. 21B

FIG. 21C

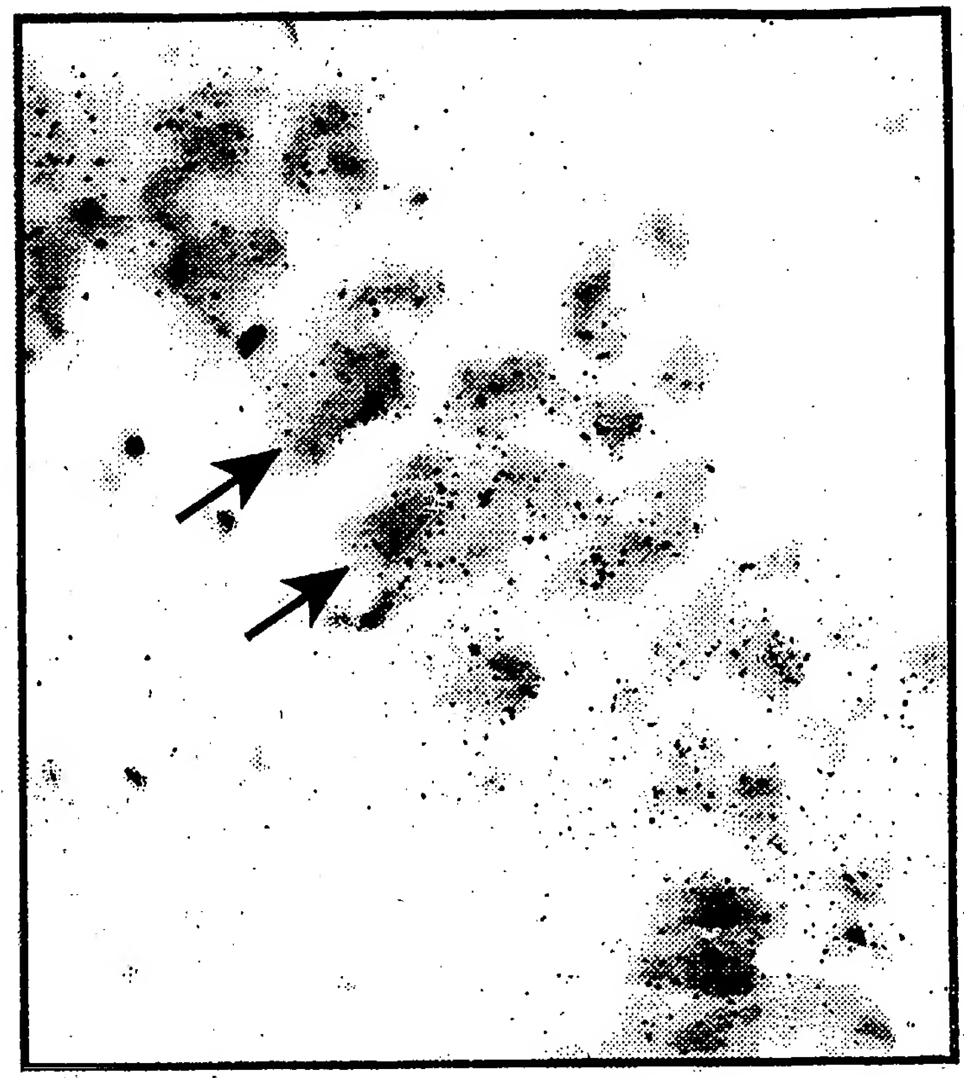


FIG. 21D

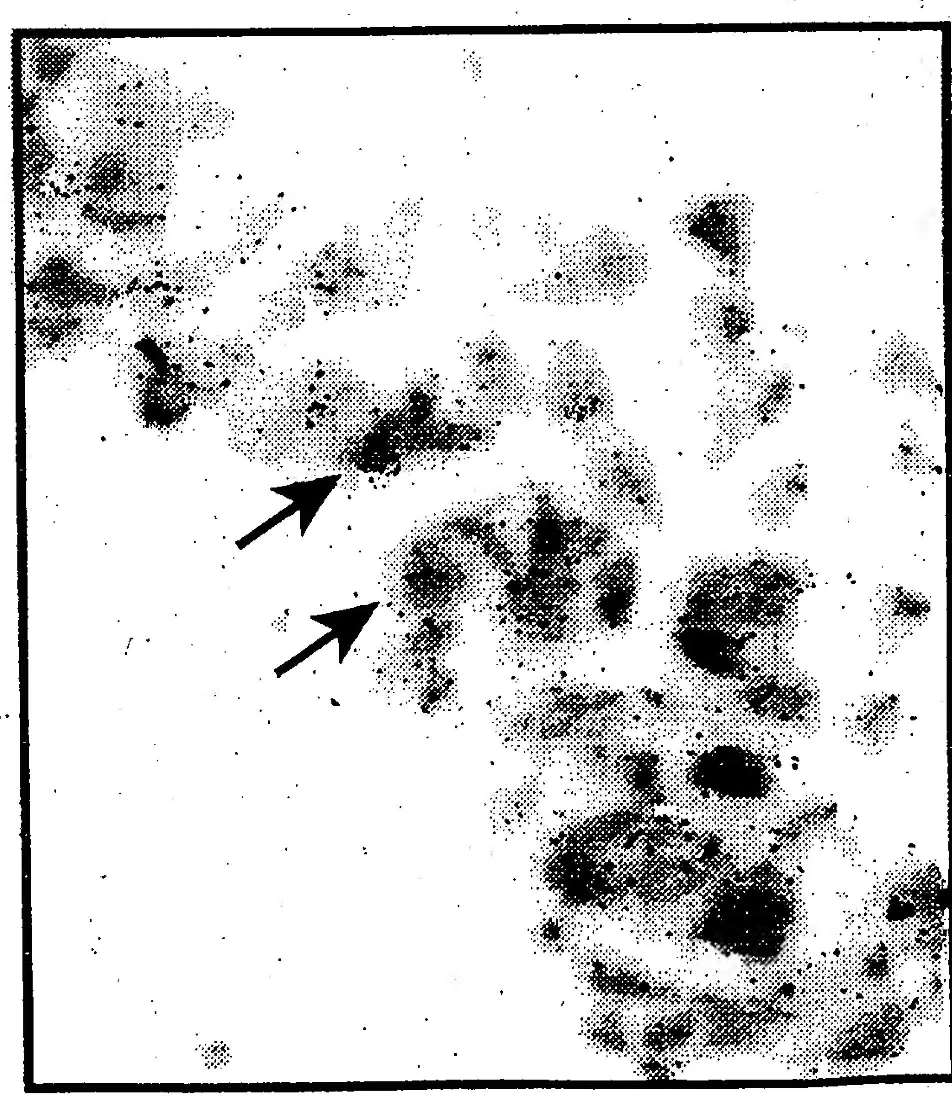


FIG. 21E

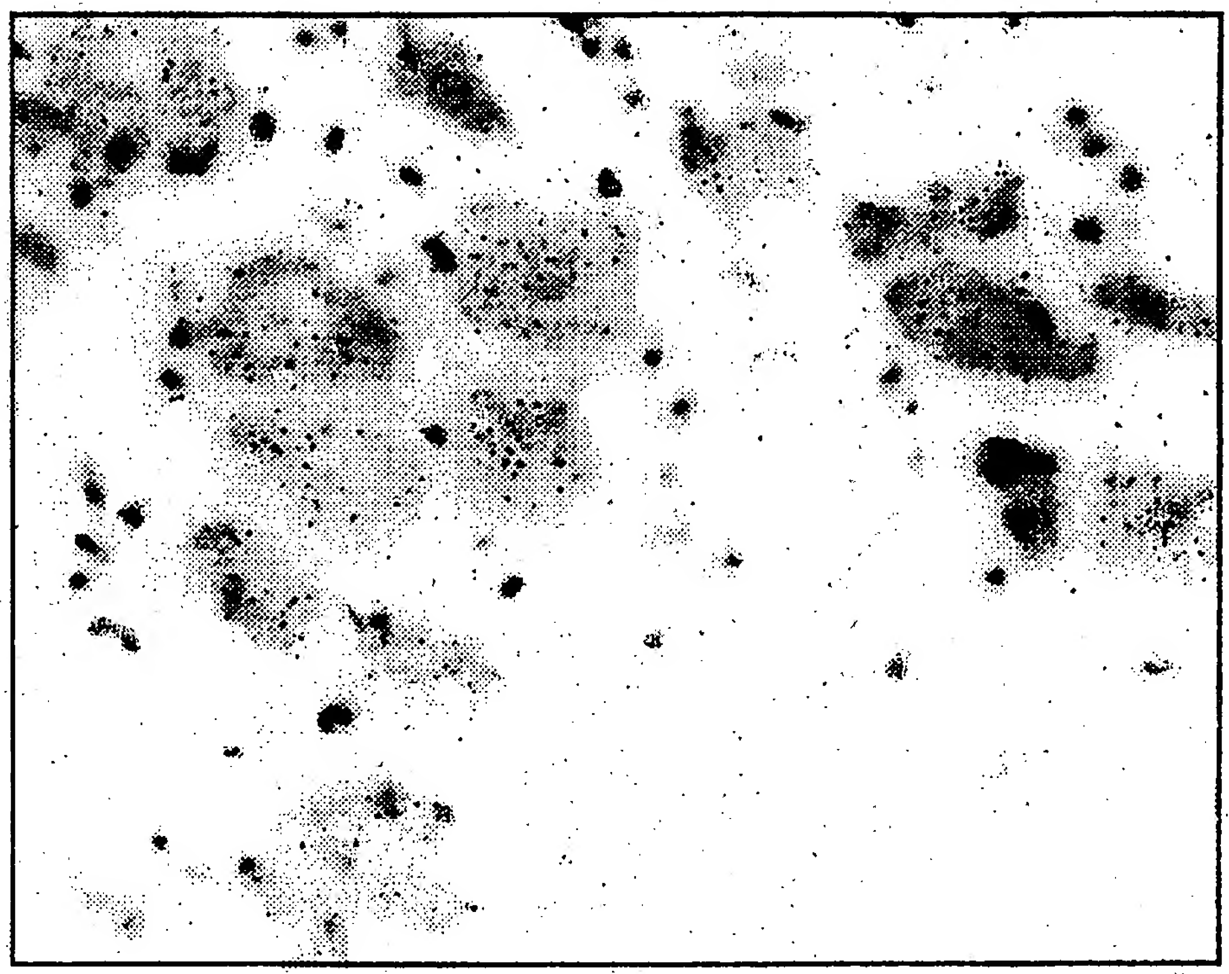
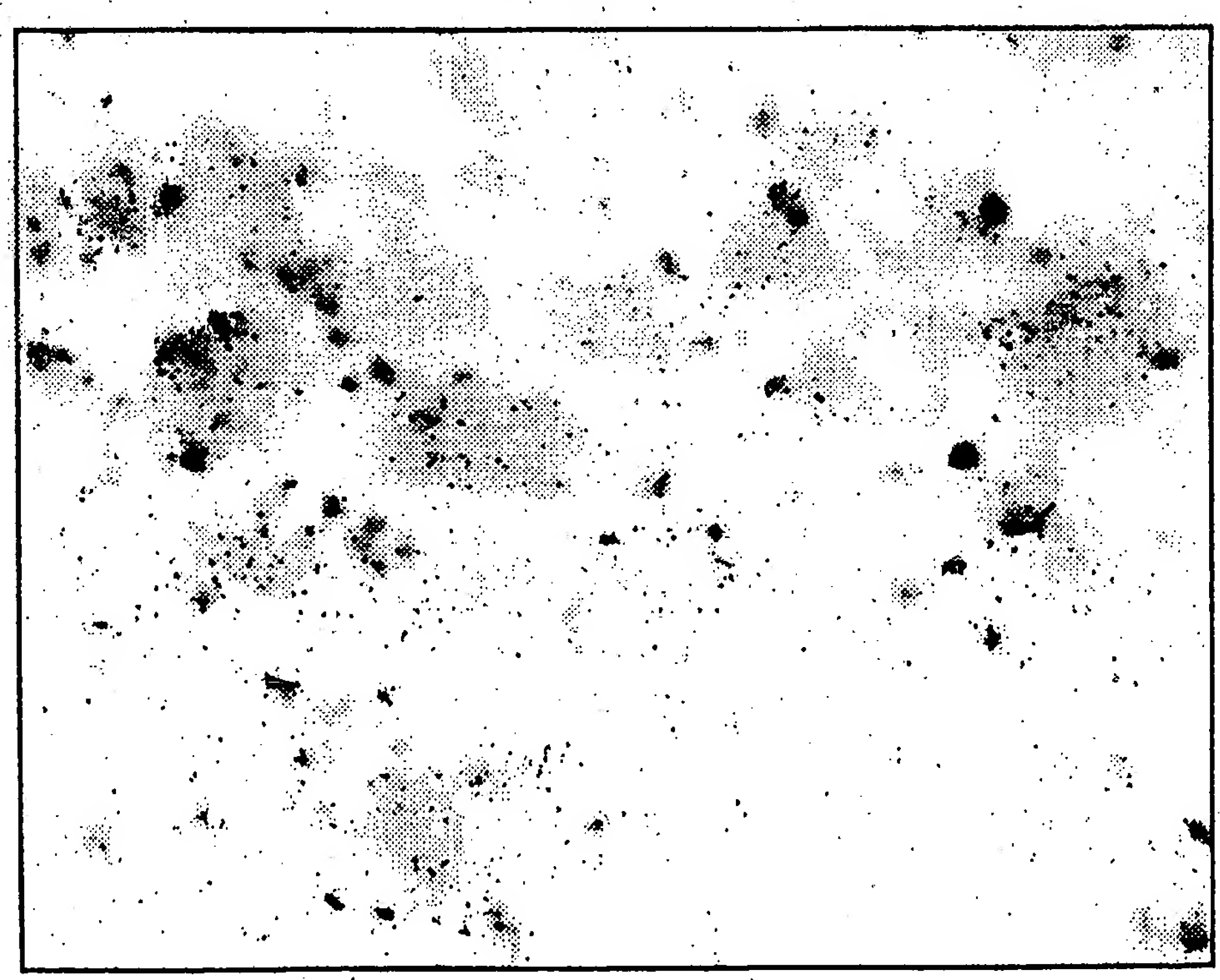


FIG. 21F



SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: SYNAPTIC PHARMACEUTICAL CORPORATION

(ii) TITLE OF INVENTION: DNA ENCODING A GABABR2 POLYPEPTIDE AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 37

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Cooper & Dunham LLP
(B) STREET: 1185 Avenue of the Americas
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: U.S.A.
(F) ZIP: 10036

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) INT'L APPL'N NUMBER:
(B) INT'L FILING DATE: 16-OCT-1998
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: White, John P.
(B) REGISTRATION NUMBER: 28,678
(C) REFERENCE/DOCKET NUMBER: 1795/54002-B-PCT/JPW/ADM

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 278-0400
(B) TELEFAX: (212) 391-0525

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3244 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GACCGCGAGG ACCGGTCCAG GCTGCGGCGG AGTCGAGGGC GAGGGAGAGG CCGCGTGAGT 180

GAGCAGAGTC CAGAGCCGTG CGCCCCCAGA ACTGCGCGTC CGCCCCGTGC ACCCCCGCGC 240

GCCATGCCCA GTTGCCCCGC GCGCTCTGCT ACGGGCCCGC TCTCCATCAT GGGCCTCATG 300

CCGCTCACCA AGGAGGTGGC CAAGGGCAGC ATCGGGCGCG GTGTGCTCCC CGCCGTGGAA 360

CTGGCCATCG AGCAGATCCG CAACGAGTCA CTCCTGCGCC CCTACTTCCT CGACCTGCGG 420

CTCTATGACA CGGAGTGCGA CAACGCAAAA GGGTTGAAAG CCTTCTACGA TGCGATAAAA 480

TACGGGCCGA ACCACTTGAT GGTGTTTGGA GGCGTCTGTC CATCCGTCAC ATCCATCATT 540

GCAGAGTCCC TCCAAGGCTG GAATCTGGTG CAGCTTTCTT TTGCTGCAAC CACGCCTGTT 600

CTAGCCGATA AGAAAAAATA CCCTTATTTC TTTCGGACCG TCCCATCAGA CAATGCGGTG 660

AATCCAGCCA TTCTGAAGTT GCTCAAGCAC TACCAGTGGA AGCGCGTGGG CACGCTGACG 720

CAAGACGTTC AGAGGTTCTC TGAGGTGCGG AATGACCTGA CTGGAGTTCT GTATGGCGAG 780

GACATTGAGA TTTCAGACAC CGAGAGCTTC TCCAACGATC CCTGTACCAG TGTCAAAAAG 840

CTGAAGGGGA ATGATGTGCG GATCATCCTT GGCCAGTTTG ACCAGAATAT GGCAGCAAAA 900

GTGTTCTGTT GTGCATACGA GGAGAACATG TATGGTAGTA AATATCAGTG GATCATTCCG 960

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CTCCGGAAGA ATCTGCTTGC TGCCATGGAG GGCTACATTG GCGTGGATTT CGAGCCCCTG 1080

AGCTCCAAGC AGATCAAGAC CATCTCAGGA AAGACTCCAC AGCAGTATGA GAGAGAGTAC 1140

AACAACAAGC GGTCAGGCGT GGGGCCCAGC AAGTTCCACG GGTACGCCTA CGATGGCATC 1200

TGGGTCATCG CCAAGACACT GCAGAGGGCC ATGGAGACAC TGCATGCCAG CAGCCGGCAC 1260

CAGCGGATCC AGGACTTCAA CTACACGGAC CACACGCTGG GCAGGATCAT CCTCAATGCC 1320

ATGAACGAGA CCAACTTCTT CGGGGTCACG GGTCAAGTTG TATTCCGGAA TGGGGAGAGA 1380

ATGGGGACCA TTAAATTTAC TCAATTTCAA GACAGCAGGG AGGTGAAGGT GGGAGAGTAC 1440

AACGCTGTGG CCGACACACT GGAGATCATC AATGACACCA TCAGGTTCCA AGGATCCGAA 1500

CCACCAAAAG ACAAGACCAT CATCCTGGAG CAGCTGCGGA AGATCTCCCT ACCTCTCTAC 1560

AGCATCCTCT CTGCCCTCAC CATCCTCGGG ATGATCATGG CCAGTGCTTT TCTCTTCTTC 1620

AACATCAAGA ACCGGAATCA GAAGCTCATA AAGATGTCGA GTCCATACAT GAACAACCTT 1680

ATCATCCTTG GAGGGATGCT TTCCTATGCT TCCATATTTC TCTTTGGCCT TGATGGATCC 1740

TTTGTCTCTG AAAAGACCTT TGAAACACTT TGCACCGTCA GGACCTGGAT TCTCACCGTG 1800

GGCTACACGA CCGCTTTTGG GGCCATGTTT GCAAAGACCT GGAGAGTCCA CGCCATCTTC 1860

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TGCATCATCG GGGCCGCTGT CTCCTTCCTG ACCCGGGACC AGCCCAATGT GCAGTTCTGC 2280
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CCTCGAGCCT TATTTGTGAA GTCCTTATTT CTTCACAAAG AAGAGGAACG GAAATGGGAC 3180
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AAAA 3244

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 898 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Pro Ser Cys Pro Ala Arg Ser Ala Thr Gly Pro Leu Ser Ile Met
1               5                   10                  15

Gly Leu Met Pro Leu Thr Lys Glu Val Ala Lys Gly Ser Ile Gly Arg
            20                  25                  30

Gly Val Leu Pro Ala Val Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu
        35                  40                  45

Ser Leu Leu Arg Pro Tyr Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu
    50                  55                  60

Cys Asp Asn Ala Lys Gly Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr
65                  70                  75                  80

Gly Pro Asn His Leu Met Val Phe Gly Gly Val Cys Pro Ser Val Thr
                85                  90                  95

Ser Ile Ile Ala Glu Ser Leu Gln Gly Trp Asn Leu Val Gln Leu Ser
            100                 105                 110

Phe Ala Ala Thr Thr Pro Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr
        115                 120                 125

Phe Phe Arg Thr Val Pro Ser Asp Asn Ala Val Asn Pro Ala Ile Leu
    130                 135                 140

Lys Leu Leu Lys His Tyr Gln Trp Lys Arg Val Gly Thr Leu Thr Gln
145                 150                 155                 160

Asp Val Gln Arg Phe Ser Glu Val Arg Asn Asp Leu Thr Gly Val Leu
                165                 170                 175

Tyr Gly Glu Asp Ile Glu Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp
            180                 185                 190

Pro Cys Thr Ser Val Lys Lys Leu Lys Gly Asn Asp Val Arg Ile Ile
        195                 200                 205

Leu Gly Gln Phe Asp Gln Asn Met Ala Ala Lys Val Phe Cys Cys Ala
    210                 215                 220

Tyr Glu Glu Asn Met Tyr Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly
225                 230                 235                 240

Trp Tyr Glu Pro Ser Trp Trp Glu Gln Val His Thr Glu Ala Asn Ser
                245                 250                 255

Ser Arg Cys Leu Arg Lys Asn Leu Leu Ala Ala Met Glu Gly Tyr Ile
            260                 265                 270

Gly Val Asp Phe Glu Pro Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser
        275                 280                 285
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Gly Lys Thr Pro Gln Gln Tyr Glu Arg Glu Tyr Asn Asn Lys Arg Ser
    290                 295                 300

Gly Val Gly Pro Ser Lys Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp
305                 310                 315                 320

Val Ile Ala Lys Thr Leu Gln Arg Ala Met Glu Thr Leu His Ala Ser
                325                 330                 335

Ser Arg His Gln Arg Ile Gln Asp Phe Asn Tyr Thr Asp His Thr Leu
            340                 345                 350

Gly Arg Ile Ile Leu Asn Ala Met Asn Glu Thr Asn Phe Phe Gly Val
        355                 360                 365

Thr Gly Gln Val Val Phe Arg Asn Gly Glu Arg Met Gly Thr Ile Lys
    370                 375                 380

Phe Thr Gln Phe Gln Asp Ser Arg Glu Val Lys Val Gly Glu Tyr Asn
385                 390                 395                 400

Ala Val Ala Asp Thr Leu Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln
                405                 410                 415

Gly Ser Glu Pro Pro Lys Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg
            420                 425                 430

Lys Ile Ser Leu Pro Leu Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu
        435                 440                 445

Gly Met Ile Met Ala Ser Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg
    450                 455                 460

Asn Gln Lys Leu Ile Lys Met Ser Ser Pro Tyr Met Asn Asn Leu Ile
465                 470                 475                 480

Ile Leu Gly Gly Met Leu Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu
                485                 490                 495

Asp Gly Ser Phe Val Ser Glu Lys Thr Phe Glu Thr Leu Cys Thr Val
            500                 505                 510

Arg Thr Trp Ile Leu Thr Val Gly Tyr Thr Thr Ala Phe Gly Ala Met
        515                 520                 525

Phe Ala Lys Thr Trp Arg Val His Ala Ile Phe Lys Asn Val Lys Met
    530                 535                 540

Lys Lys Lys Ile Ile Lys Asp Gln Lys Leu Leu Val Ile Val Gly Gly
545                 550                 555                 560

Met Leu Leu Ile Asp Leu Cys Ile Leu Ile Cys Trp Gln Ala Val Asp
                565                 570                 575

Pro Leu Arg Arg Thr Val Glu Lys Tyr Ser Met Glu Pro Asp Pro Ala
            580                 585                 590

Gly Arg Asp Ile Ser Ile Arg Pro Leu Leu Glu His Cys Glu Asn Thr
        595                 600                 605
```

```
His Met Thr Ile Trp Leu Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu
    610                 615                 620

Met Leu Phe Gly Cys Phe Leu Ala Trp Glu Thr Arg Asn Val Ser Ile
625                 630                 635                 640

Pro Ala Leu Asn Asp Ser Lys Tyr Ile Gly Met Ser Val Tyr Asn Val
                645                 650                 655

Gly Ile Met Cys Ile Ile Gly Ala Ala Val Ser Phe Leu Thr Arg Asp
            660                 665                 670

Gln Pro Asn Val Gln Phe Cys Ile Val Ala Leu Val Ile Ile Phe Cys
        675                 680                 685

Ser Thr Ile Thr Leu Cys Leu Val Phe Val Pro Lys Leu Ile Thr Leu
    690                 695                 700

Arg Thr Asn Pro Asp Ala Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr
705                 710                 715                 720

Gln Asn Gln Lys Lys Glu Asp Ser Lys Thr Ser Thr Ser Val Thr Ser
                725                 730                 735

Val Asn Gln Ala Ser Thr Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn
            740                 745                 750

His Arg Leu Arg Met Lys Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu
        755                 760                 765

Val Thr Met Gln Leu Gln Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys
    770                 775                 780

Gln Asn His Tyr Gln Glu Leu Asn Asp Ile Leu Asn Leu Gly Asn Phe
785                 790                 795                 800

Thr Glu Ser Thr Asp Gly Gly Lys Ala Ile Leu Lys Asn His Leu Asp
                805                 810                 815

Gln Asn Pro Gln Leu Gln Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys
            820                 825                 830

Lys Asp Pro Ile Glu Asp Ile Asn Ser Pro Glu His Ile Gln Arg Arg
        835                 840                 845

Leu Ser Leu Gln Leu Pro Ile Leu His His Ala Tyr Leu Pro Ser Ile
    850                 855                 860

Gly Gly Val Asp Ala Ser Cys Val Ser Pro Cys Val Ser Pro Thr Ala
865                 870                 875                 880

Ser Pro Arg His Arg His Val Pro Pro Ser Phe Arg Val Met Val Ser
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Gly Leu
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2652 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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CTGGACCTGC GACTCTATGA CACCGAGTGT GACAATGCAA AGGGACTGAA AGCCTTCTAT     180
GACGCAATAA AGTATGGGCT GAACCATTTG ATGGTGTTTG GAGGCGTCTG TCCGTCTGTC     240
ACATCTATTA TCGCGGAGTC CCTCCAAGGC TGGAATCTGG TGCAGCTTTC CTTCGCCGCC     300
ACCACGCCTG TTCTTGCGGA TAAGAAGAAG TACCCGTATT TCTTCCGGAC GGTGCCGTCA     360
GACAACGCGG TGAACCCCGC CATCCTGAAG CTCCTGAAGC ACTTCCGCTG GCGGCGTGTG     420
GGCACACTCA CGCAGGACGT GCAGCGCTTC TCCGAGGTGA GGAATGACCT GACTGGGGTT     480
CTGTATGGGG AAGATATTGA GATCTCAGAC ACAGAGAGTT TCTCCAATGA TCCCTGCACC     540
AGCGTCAAAA AGCTCAAGGG GAATGACGTG CGGATCATCC TTGGCCAGTT TGACCAGAAT     600
ATGGCAGCAA AAGTCTTCTG TTGTGCCTTC GAGGAGAGCA TGTTTGGCAG CAAGTACCAG     660
TGGATCATCC CGGGATGGTA CGAGCCTGCG TGGTGGGAGC AGGTGCATGT GGAGGCCAAT     720
TCCTCACGCT GCCTGCGCAG AAGCCTCCTG GCTGCCATGG AAGGTTACAT CGGAGTGGAC     780
TTTGAGCCCC TGAGCTCCAA ACAAATCAAG ACCATCTCAG GGAAGACTCC ACAGCAGTAT     840
GAAAGAGAGT ACAACAGCAA ACGTTCAGGC GTGGGGCCCA GCAAGTTCCA TGGGTACGCC     900
TACGATGGGA TCTGGGTCAT CGCCAAGACC CTACAGAGGG CCATGGAGAC ACTGCATGCC     960
AGTAGCAGGC ACCAGCGGAT CCAGGACTTC AACTACACAG ACCACACGCT GGGCAAAATC    1020
ATCCTCAATG CCATGAACGA GACCAACTTC TTCGGGGTCA CGGGTCAAGT TGTGTTCCGG    1080
AACGGGGAGA GAATGGGAAC CATTAAATTT ACTCAATTTC AAGACAGCAG AGAGGTGAAG    1140
GTCGGCGAAT ACAACGCGGT GGCTGACACA CTGGAGATCA TCAATGACAC CATAAGGTTC    1200
CAGGGGTCCG AGCCACCCAA GGACAAGACC ATCATTCTGG AGCAGCTTCG GAAGATCTCG    1260
CTTCCACTGT ATAGCATCCT GTCCGCTCTC ACCATCCTCG GCATGATCAT GGCCAGCGCC    1320
TTCCTCTTCT TCAACATCAA GAACCGGAAC CAAAAGCTGA TTAAGATGTC AAGCCCCTAC    1380
```

```
ATGAACAACC TCATCATCCT GGGAGGAATG CTGTCCTATG CATCCATCTT CCTCTTTGGC    1440
CTCGATGGGT CCTTCGTCTC AGAAAAGACC TTTGAAACAC TCTGCACGGT CCGGACCTGG    1500
ATTCTCACCG TGGGCTACAC AACTGCCTTT GGGGCCATGT TTGCAAAGAC CTGGAGGGTC    1560
CATGCCATCT TCAAAAATGT GAAGATGAAG AAGAAGATCA TCAAAGACCA GAAGCTGCTT    1620
GTGATTGTGG GGGGCATGCT GCTCATCGAC CTGTGCATCC TGATCTGTTG GCAGGCTGTG    1680
GACCCCCTGC GGAGGACAGT AGAGAGGTAC AGCATGGAGC CGGACCCAGC AGGCCGGGAC    1740
ATCTCCATCC GCCCATTGCT GGAACACTGC GAAAACACCC ACATGACCAT CTGGCTTGGC    1800
ATTGTCTACG CCTACAAGGG GCTCCTCATG CTATTCGGTT GTTTCTTGGC ATGGGAAACC    1860
CGCAATGTGA GCATCCCTGC CCTCAACGAC AGCAAGTACA TCGGCATGAG TGTGTACAAT    1920
GTGGGGATCA TGTGCATCAT CGGGGCTGCT GTCTCCTTCC TGACGCGTGA CCAGCCCAAC    1980
GTGCAGTTCT GCATCGTGGC CCTGGTCATC ATCTTCTGCA GCACCATCAC TCTCTGCCTG    2040
GTGTTTGTGC CAAAGCTCAT TACTCTGAGG ACAAACCCTG ACGCAGCCAC TCAGAACAGG    2100
CGGTTCCAGT TCACACAGAA CCAGAAGAAA GAAGATTCGA AGACCTCCAC TTCAGTCACC    2160
AGCGTGAACC AGGCGAGCAC GTCACGCCTG GAGGGACTGC AGTCAGAAAA CCACCGCCTT    2220
CGAATGAAGA TCACAGAGCT GGACAAAGAC TTGGAAGAAG TCACCATGCA GCTACAAGAC    2280
ACACCAGAGA AGACCACATA CATCAAACAG AATCACTACC AAGAGCTCAA CGACATCCTC    2340
AGCTTGGGCA ACTTCACAGA GAGCACAGAT GGAGGAAAGG CCATTCTAAA AAATCACCTC    2400
GATCAAAACC CCCAGCTCCA GTGGAACACG ACAGAGCCCT CAAGAACATG CAAAGACCCC    2460
ATAGAAGACA TCAACTCCCC GGAGCACATC CAGCGCCGGC TGTCGCTCCA GCTCCCCATC    2520
CTTCACCACG CCTACCTCCC ATCCATCGGA GGCGTGGATG CCAGCTGCGT CAGCCCCTGT    2580
GTCAGCCCTA CCGCCAGCCC TCGCCACAGA CACGTACCAC CCTCCTTCCG AGTCATGGTC    2640
TCGGGCCTGT AG                                                        2652
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 883 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Gly Leu Met Pro Leu Thr Lys Glu Val Ala Lys Gly Ser Ile Gly
1               5                   10                  15

Arg Gly Val Leu Pro Ala Val Glu Leu Ala Ile Glu Gln Ile Arg Asn
            20                  25                  30

Glu Ser Leu Leu Arg Pro Tyr Phe Leu Asp Leu Arg Leu Tyr Asp Thr
        35                  40                  45

Glu Cys Asp Asn Ala Lys Gly Leu Lys Ala Phe Tyr Asp Ala Ile Lys
    50                  55                  60

Tyr Gly Leu Asn His Leu Met Val Phe Gly Gly Val Cys Pro Ser Val
65                  70                  75                  80

Thr Ser Ile Ile Ala Glu Ser Leu Gln Gly Trp Asn Leu Val Gln Leu
                85                  90                  95

Ser Phe Ala Ala Thr Thr Pro Val Leu Ala Asp Lys Lys Lys Tyr Pro
            100                 105                 110

Tyr Phe Phe Arg Thr Val Pro Ser Asp Asn Ala Val Asn Pro Ala Ile
        115                 120                 125

Leu Lys Leu Leu Lys His Phe Arg Trp Arg Arg Val Gly Thr Leu Thr
    130                 135                 140

Gln Asp Val Gln Arg Phe Ser Glu Val Arg Asn Asp Leu Thr Gly Val
145                 150                 155                 160

Leu Tyr Gly Glu Asp Ile Glu Ile Ser Asp Thr Glu Ser Phe Ser Asn
                165                 170                 175

Asp Pro Cys Thr Ser Val Lys Lys Leu Lys Gly Asn Asp Val Arg Ile
            180                 185                 190

Ile Leu Gly Gln Phe Asp Gln Asn Met Ala Ala Lys Val Phe Cys Cys
        195                 200                 205

Ala Phe Glu Glu Ser Met Phe Gly Ser Lys Tyr Gln Trp Ile Ile Pro
    210                 215                 220

Gly Trp Tyr Glu Pro Ala Trp Trp Glu Gln Val His Val Glu Ala Asn
225                 230                 235                 240

Ser Ser Arg Cys Leu Arg Arg Ser Leu Leu Ala Ala Met Glu Gly Tyr
                245                 250                 255

Ile Gly Val Asp Phe Glu Pro Leu Ser Ser Lys Gln Ile Lys Thr Ile
            260                 265                 270

Ser Gly Lys Thr Pro Gln Gln Tyr Glu Arg Glu Tyr Asn Ser Lys Arg
        275                 280                 285

Ser Gly Val Gly Pro Ser Lys Phe His Gly Tyr Ala Tyr Asp Gly Ile
    290                 295                 300

Trp Val Ile Ala Lys Thr Leu Gln Arg Ala Met Glu Thr Leu His Ala
305                 310                 315                 320
```

```
Ser Ser Arg His Gln Arg Ile Gln Asp Phe Asn Tyr Thr Asp His Thr
                325                 330                 335

Leu Gly Lys Ile Ile Leu Asn Ala Met Asn Glu Thr Asn Phe Phe Gly
            340                 345                 350

Val Thr Gly Gln Val Val Phe Arg Asn Gly Glu Arg Met Gly Thr Ile
        355                 360                 365

Lys Phe Thr Gln Phe Gln Asp Ser Arg Glu Val Lys Val Gly Glu Tyr
    370                 375                 380

Asn Ala Val Ala Asp Thr Leu Glu Ile Ile Asn Asp Thr Ile Arg Phe
385                 390                 395                     400

Gln Gly Ser Glu Pro Pro Lys Asp Lys Thr Ile Ile Leu Glu Gln Leu
                405                 410                 415

Arg Lys Ile Ser Leu Pro Leu Tyr Ser Ile Leu Ser Ala Leu Thr Ile
            420                 425                 430

Leu Gly Met Ile Met Ala Ser Ala Phe Leu Phe Phe Asn Ile Lys Asn
        435                 440                 445

Arg Asn Gln Lys Leu Ile Lys Met Ser Ser Pro Tyr Met Asn Asn Leu
    450                 455                 460

Ile Ile Leu Gly Gly Met Leu Ser Tyr Ala Ser Ile Phe Leu Phe Gly
465                 470                 475                     480

Leu Asp Gly Ser Phe Val Ser Glu Lys Thr Phe Glu Thr Leu Cys Thr
                485                 490                 495

Val Arg Thr Trp Ile Leu Thr Val Gly Tyr Thr Thr Ala Phe Gly Ala
            500                 505                 510

Met Phe Ala Lys Thr Trp Arg Val His Ala Ile Phe Lys Asn Val Lys
        515                 520                 525

Met Lys Lys Lys Ile Ile Lys Asp Gln Lys Leu Leu Val Ile Val Gly
    530                 535                 540

Gly Met Leu Leu Ile Asp Leu Cys Ile Leu Ile Cys Trp Gln Ala Val
545                 550                 555                     560

Asp Pro Leu Arg Arg Thr Val Glu Arg Tyr Ser Met Glu Pro Asp Pro
                565                 570                 575

Ala Gly Arg Asp Ile Ser Ile Arg Pro Leu Leu Glu His Cys Glu Asn
            580                 585                 590

Thr His Met Thr Ile Trp Leu Gly Ile Val Tyr Ala Tyr Lys Gly Leu
        595                 600                 605

Leu Met Leu Phe Gly Cys Phe Leu Ala Trp Glu Thr Arg Asn Val Ser
    610                 615                 620

Ile Pro Ala Leu Asn Asp Ser Lys Tyr Ile Gly Met Ser Val Tyr Asn
625                 630                 635                     640
```

```
Val Gly Ile Met Cys Ile Ile Gly Ala Ala Val Ser Phe Leu Thr Arg
                645                 650                 655

Asp Gln Pro Asn Val Gln Phe Cys Ile Val Ala Leu Val Ile Ile Phe
            660                 665                 670

Cys Ser Thr Ile Thr Leu Cys Leu Val Phe Val Pro Lys Leu Ile Thr
        675                 680                 685

Leu Arg Thr Asn Pro Asp Ala Ala Thr Gln Asn Arg Arg Phe Gln Phe
    690                 695                 700

Thr Gln Asn Gln Lys Lys Glu Asp Ser Lys Thr Ser Thr Ser Val Thr
705                 710                 715                 720

Ser Val Asn Gln Ala Ser Thr Ser Arg Leu Glu Gly Leu Gln Ser Glu
                725                 730                 735

Asn His Arg Leu Arg Met Lys Ile Thr Glu Leu Asp Lys Asp Leu Glu
            740                 745                 750

Glu Val Thr Met Gln Leu Gln Asp Thr Pro Glu Lys Thr Thr Tyr Ile
        755                 760                 765

Lys Gln Asn His Tyr Gln Glu Leu Asn Asp Ile Leu Ser Leu Gly Asn
    770                 775                 780

Phe Thr Glu Ser Thr Asp Gly Gly Lys Ala Ile Leu Lys Asn His Leu
785                 790                 795                 800

Asp Gln Asn Pro Gln Leu Gln Trp Asn Thr Thr Glu Pro Ser Arg Thr
                805                 810                 815

Cys Lys Asp Pro Ile Glu Asp Ile Asn Ser Pro Glu His Ile Gln Arg
            820                 825                 830

Arg Leu Ser Leu Gln Leu Pro Ile Leu His His Ala Tyr Leu Pro Ser
        835                 840                 845

Ile Gly Gly Val Asp Ala Ser Cys Val Ser Pro Cys Val Ser Pro Thr
850                 855                 860

Ala Ser Pro Arg His Arg His Val Pro Pro Ser Phe Arg Val Met Val
865                 870                 875                 880

Ser Gly Leu
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 45 base pairs
  (B) TYPE: nucleic acid
  (C) STRANDEDNESS: single
  (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGGGATGCTT TCCTATGCTT CCATATTTCT CTTTGGCCTT GATGG 45

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAATGTGCAG TTCTGCATCG TGGCTCTGGT CATCATCTTC TGCAG 45

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTTCTAGGCC TGTACGGAAG TGTT 24

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTGTGGTTT GTCCAAACTC ATCAAT 26

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGGATGAGTG TCTACAACGT GGGG 24

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGCGTTGCTG CATCTGGGTT TGTTCT 26

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATCTCCCTAC CTCTCTACAG CATCCT 26

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CAGGTCCTGA CGGTGCAAAG TGTTTC 26

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGACGCAAGA CGTTCAGAGG TTCTCT 26

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TGTAGCCTTC CATGGCAGCA AGCAGA 26

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AGAGAACCTC TGAACGTCTT GCGTCA 26

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGCTCTGTTG TGTTCCACTG TAGCTG 26

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TCATGCCGCT CACCAAGGAG GTGGCC 26

2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGCCACCTCC TTGGTGAGCG GCATGA 26

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TGAGTGAGCA GAGTCCAGAG CCGT 24

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATGGATGGGA GGTAGGCGTG GTGGAG 26

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CTCTCTGCCC TCACCATCCT CGGGAT 26

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GACTCCGGCT CGAATACCAG GCAGAG 26

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CCATGTTTGC AAAGACCTGG AGGGTCC 27

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGTCACGCGT CAGGAAAGAG ACAGCAG 27

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AAGCTTCTAG AGATCCCTCG ACCTC 25

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AGGCGCAGAA CTGGTAGGTA TGGAA 25

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTTCTAGGCC TGTACGGAAG TGTTA 25

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GTTGTGGTTT GTCCAAACTC ATCAATG 27

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CTGCTGTCTC TTTCCTGACG CGTGACC 27

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CCAAGCTTCT AATACGACTC ACTATAGGGG AGACCATGGG CCCGGGGGGA CCCTGTACC 59

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTCACTT GTAAAGCAAA TGTACTCGAC TCC 63

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CGCGGATCCA TTATGTCTGC ACTCCGAAGG AAATTTG 37

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CGCGAATTCT TATGTGAAGC GATCAGAGTT CATTTTTC 38

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GCGGGATCCG CTATGGCTGG TGATTCTAGG AATG 34

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CCGGAATTCC CCTCACACCG AGCCCCTGG 29

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 844 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Met Gly Pro Gly Gly Pro Cys Thr Pro Val Gly Trp Pro Leu Pro Leu
1               5                   10                  15

Leu Leu Val Met Ala Ala Gly Val Ala Pro Val Trp Ala Ser His Ser
            20                  25                  30

Pro His Leu Pro Arg Pro His Pro Arg Val Pro Pro His Pro Ser Ser
        35                  40                  45

Glu Arg Arg Ala Val Tyr Ile Gly Ala Leu Phe Pro Met Ser Gly Gly
    50                  55                  60

Trp Pro Gly Gly Gln Ala Cys Gln Pro Ala Val Glu Met Ala Leu Glu
65                  70                  75                  80

Asp Val Asn Ser Arg Arg Asp Ile Leu Pro Asp Tyr Glu Leu Lys Leu
                85                  90                  95

Ile His His Asp Ser Lys Cys Asp Pro Gly Gln Ala Thr Lys Tyr Leu
            100                 105                 110

Tyr Glu Leu Leu Tyr Asn Asp Pro Ile Lys Ile Ile Leu Met Pro Gly
        115                 120                 125

Cys Ser Ser Val Ser Thr Leu Val Ala Glu Ala Ala Arg Met Trp Asn
    130                 135                 140

Leu Ile Val Leu Ser Tyr Gly Ser Ser Ser Pro Ala Leu Ser Asn Arg
145                 150                 155                 160

Gln Arg Phe Pro Thr Phe Phe Arg Thr His Pro Ser Ala Thr Leu His
                165                 170                 175

Asn Pro Thr Arg Val Lys Leu Phe Glu Lys Trp Gly Trp Lys Lys Ile
            180                 185                 190

Ala Thr Ile Gln Gln Thr Thr Glu Val Phe Thr Ser Thr Leu Asp Asp
        195                 200                 205

Leu Glu Glu Arg Val Lys Glu Ala Gly Ile Glu Ile Thr Phe Arg Gln
    210                 215                 220

Ser Phe Phe Ser Asp Pro Ala Val Pro Val Lys Asn Leu Lys Arg Gln
225                 230                 235                 240

Asp Ala Arg Ile Ile Val Gly Leu Phe Tyr Glu Thr Glu Ala Arg Lys
                245                 250                 255

Val Phe Cys Glu Val Tyr Lys Glu Arg Leu Phe Gly Lys Lys Tyr Val
            260                 265                 270

Trp Phe Leu Ile Gly Trp Tyr Ala Asp Asn Trp Phe Lys Thr Tyr Asp
        275                 280                 285

Pro Ser Ile Asn Cys Thr Val Glu Glu Met Thr Glu Ala Val Glu Gly
    290                 295                 300
```

His Ile Thr Thr Glu Ile Val Met Leu Asn Pro Ala Asn Thr Arg Ser
305 310 315 320

Ile Ser Asn Met Thr Ser Gln Glu Phe Val Glu Lys Leu Thr Lys Arg
325 330 335

Leu Lys Arg His Pro Glu Glu Thr Gly Gly Phe Gln Glu Ala Pro Leu
340 345 350

Ala Tyr Asp Ala Ile Trp Ala Leu Ala Leu Ala Leu Asn Lys Thr Ser
355 360 365

Gly Gly Gly Gly Arg Ser Gly Val Arg Leu Glu Asp Phe Asn Tyr Asn
370 375 380

Asn Gln Thr Ile Thr Asp Gln Ile Tyr Arg Ala Met Asn Ser Ser Ser
385 390 395 400

Phe Glu Gly Val Ser Gly His Val Val Phe Asp Ala Ser Gly Ser Arg
405 410 415

Met Ala Trp Thr Leu Ile Glu Gln Leu Gln Gly Gly Ser Tyr Lys Lys
420 425 430

Ile Gly Tyr Tyr Asp Ser Thr Lys Asp Asp Leu Ser Trp Ser Lys Thr
435 440 445

Asp Lys Trp Ile Gly Gly Ser Pro Pro Ala Asp Gln Thr Leu Val Ile
450 455 460

Lys Thr Phe Arg Phe Leu Ser Gln Lys Leu Phe Ile Ser Val Ser Val
465 470 475 480

Leu Ser Ser Leu Gly Ile Val Leu Ala Val Val Cys Leu Ser Phe Asn
485 490 495

Ile Tyr Asn Ser His Val Arg Tyr Ile Gln Asn Ser Gln Pro Asn Leu
500 505 510

Asn Asn Leu Thr Ala Val Gly Cys Ser Leu Ala Leu Ala Ala Val Phe
515 520 525

Pro Leu Gly Leu Asp Gly Tyr His Ile Gly Arg Ser Gln Phe Pro Phe
530 535 540

Val Cys Gln Ala Arg Leu Trp Leu Leu Gly Leu Gly Phe Ser Leu Gly
545 550 555 560

Tyr Gly Ser Met Phe Thr Lys Ile Trp Trp Val His Thr Val Phe Thr
565 570 575

Lys Lys Glu Glu Lys Lys Glu Trp Arg Lys Thr Leu Glu Pro Trp Lys
580 585 590

Leu Tyr Ala Thr Val Gly Leu Leu Val Gly Met Asp Val Leu Thr Leu
595 600 605

Ala Ile Trp Gln Ile Val Asp Pro Leu His Arg Thr Ile Glu Thr Phe
610 615 620

```
Ala Lys Glu Glu Pro Lys Glu Asp Ile Asp Val Ser Ile Leu Pro Gln
625                 630                 635                 640

Leu Glu His Cys Ser Ser Lys Lys Met Asn Thr Trp Leu Gly Ile Phe
                645                 650                 655

Tyr Gly Tyr Lys Gly Leu Leu Leu Leu Leu Gly Ile Phe Leu Ala Tyr
            660                 665                 670

Glu Thr Lys Ser Val Ser Thr Glu Lys Ile Asn Asp His Arg Ala Val
        675                 680                 685

Gly Met Ala Ile Tyr Asn Val Ala Val Leu Cys Leu Ile Thr Ala Pro
    690                 695                 700

Val Thr Met Ile Leu Ser Ser Gln Gln Asp Ala Ala Phe Ala Phe Ala
705                 710                 715                 720

Ser Leu Ala Ile Val Phe Ser Ser Tyr Ile Thr Leu Val Val Leu Phe
                725                 730                 735

Val Pro Lys Met Arg Arg Leu Ile Thr Arg Gly Glu Trp Gln Ser Glu
            740                 745                 750

Thr Gln Asp Thr Met Lys Thr Gly Ser Ser Thr Asn Asn Asn Glu Glu
        755                 760                 765

Glu Lys Ser Arg Leu Leu Glu Lys Glu Asn Arg Glu Leu Glu Lys Ile
    770                 775                 780

Ile Ala Glu Lys Glu Glu Arg Val Ser Glu Leu Arg His Gln Leu Gln
785                 790                 795                 800

Ser Arg Gln Gln Leu Arg Ser Arg Arg His Pro Pro Thr Pro Pro Asp
                805                 810                 815

Pro Ser Gly Gly Leu Pro Arg Gly Pro Ser Glu Pro Pro Asp Arg Leu
            820                 825                 830

Ser Cys Asp Gly Ser Arg Val His Leu Leu Tyr Lys
        835                 840
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 883 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
Met Gly Leu Met Pro Leu Thr Lys Glu Val Ala Lys Gly Ser Ile Gly
1               5                   10                  15
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Arg Gly Val Leu Pro Ala Val Glu Leu Ala Ile Glu Gln Ile Arg Asn
            20                  25                  30

Glu Ser Leu Leu Arg Pro Tyr Phe Leu Asp Leu Arg Leu Tyr Asp Thr
        35                  40                  45

Glu Cys Asp Asn Ala Lys Gly Leu Lys Ala Phe Tyr Asp Ala Ile Lys
    50                  55                  60

Tyr Gly Leu Asn His Leu Met Val Phe Gly Gly Val Cys Pro Ser Val
65                  70                  75                  80

Thr Ser Ile Ile Ala Glu Ser Leu Gln Gly Trp Asn Leu Val Gln Leu
                85                  90                  95

Ser Phe Ala Ala Thr Thr Pro Val Leu Ala Asp Lys Lys Lys Tyr Pro
            100                 105                 110

Tyr Phe Phe Arg Thr Val Pro Ser Asp Asn Ala Val Asn Pro Ala Ile
        115                 120                 125

Leu Lys Leu Leu Lys His Phe Arg Trp Arg Arg Val Gly Thr Leu Thr
    130                 135                 140

Gln Asp Val Gln Arg Phe Ser Glu Val Arg Asn Asp Leu Thr Gly Val
145                 150                 155                 160

Leu Tyr Gly Glu Asp Ile Glu Ile Ser Asp Thr Glu Ser Phe Ser Asn
                165                 170                 175

Asp Pro Cys Thr Ser Val Lys Lys Leu Lys Gly Asn Asp Val Arg Ile
            180                 185                 190

Ile Leu Gly Gln Phe Asp Gln Asn Met Ala Ala Lys Val Phe Cys Cys
        195                 200                 205

Ala Phe Glu Glu Ser Met Phe Gly Ser Lys Tyr Gln Trp Ile Ile Pro
    210                 215                 220

Gly Trp Tyr Glu Pro Ala Trp Trp Glu Gln Val His Val Glu Ala Asn
225                 230                 235                 240

Ser Ser Arg Cys Leu Arg Arg Ser Leu Leu Ala Ala Met Glu Gly Tyr
                245                 250                 255

Ile Gly Val Asp Phe Glu Pro Leu Ser Ser Lys Gln Ile Lys Thr Ile
            260                 265                 270

Ser Gly Lys Thr Pro Gln Gln Tyr Glu Arg Glu Tyr Asn Ser Lys Arg
        275                 280                 285

Ser Gly Val Gly Pro Ser Lys Phe His Gly Tyr Ala Tyr Asp Gly Ile
    290                 295                 300

Trp Val Ile Ala Lys Thr Leu Gln Arg Ala Met Glu Thr Leu His Ala
305                 310                 315                 320

Ser Ser Arg His Gln Arg Ile Gln Asp Phe Asn Tyr Thr Asp His Thr
                325                 330                 335
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Leu Gly Lys Ile Ile Leu Asn Ala Met Asn Glu Thr Asn Phe Phe Gly
340 345 350

Val Thr Gly Gln Val Val Phe Arg Asn Gly Glu Arg Met Gly Thr Ile
355 360 365

Lys Phe Thr Gln Phe Gln Asp Ser Arg Glu Val Lys Val Gly Glu Tyr
370 375 380

Asn Ala Val Ala Asp Thr Leu Glu Ile Ile Asn Asp Thr Ile Arg Phe
385 390 395 400

Gln Gly Ser Glu Pro Pro Lys Asp Lys Thr Ile Ile Leu Glu Gln Leu
405 410 415

Arg Lys Ile Ser Leu Pro Leu Tyr Ser Ile Leu Ser Ala Leu Thr Ile
420 425 430

Leu Gly Met Ile Met Ala Ser Ala Phe Leu Phe Phe Asn Ile Lys Asn
435 440 445

Arg Asn Gln Lys Leu Ile Lys Met Ser Ser Pro Tyr Met Asn Asn Leu
450 455 460

Ile Ile Leu Gly Gly Met Leu Ser Tyr Ala Ser Ile Phe Leu Phe Gly
465 470 475 480

Leu Asp Gly Ser Phe Val Ser Glu Lys Thr Phe Glu Thr Leu Cys Thr
485 490 495

Val Arg Thr Trp Ile Leu Thr Val Gly Tyr Thr Thr Ala Phe Gly Ala
500 505 510

Met Phe Ala Lys Thr Trp Arg Val His Ala Ile Phe Lys Asn Val Lys
515 520 525

Met Lys Lys Lys Ile Ile Lys Asp Gln Lys Leu Leu Val Ile Val Gly
530 535 540

Gly Met Leu Leu Ile Asp Leu Cys Ile Leu Ile Cys Trp Gln Ala Val
545 550 555 560

Asp Pro Leu Arg Arg Thr Val Glu Arg Tyr Ser Met Glu Pro Asp Pro
565 570 575

Ala Gly Arg Asp Ile Ser Ile Arg Pro Leu Leu Glu His Cys Glu Asn
580 585 590

Thr His Met Thr Ile Trp Leu Gly Ile Val Tyr Ala Tyr Lys Gly Leu
595 600 605

Leu Met Leu Phe Gly Cys Phe Leu Ala Trp Glu Thr Arg Asn Val Ser
610 615 620

Ile Pro Ala Leu Asn Asp Ser Lys Tyr Ile Gly Met Ser Val Tyr Asn
625 630 635 640

Val Gly Ile Met Cys Ile Ile Gly Ala Ala Val Ser Phe Leu Thr Arg
645 650 655

Asp Gln Pro Asn Val Gln Phe Cys Ile Val Ala Leu Val Ile Ile Phe
660 665 670

Cys Ser Thr Ile Thr Leu Cys Leu Val Phe Val Pro Lys Leu Ile Thr
675 680 685

Leu Arg Thr Asn Pro Asp Ala Ala Thr Gln Asn Arg Arg Phe Gln Phe
690 695 700

Thr Gln Asn Gln Lys Lys Glu Asp Ser Lys Thr Ser Thr Ser Val Thr
705 710 715 720

Ser Val Asn Gln Ala Ser Thr Ser Arg Leu Glu Gly Leu Gln Ser Glu
725 730 735

Asn His Arg Leu Arg Met Lys Ile Thr Glu Leu Asp Lys Asp Leu Glu
740 745 750

Glu Val Thr Met Gln Leu Gln Asp Thr Pro Glu Lys Thr Thr Tyr Ile
755 760 765

Lys Gln Asn His Tyr Gln Glu Leu Asn Asp Ile Leu Ser Leu Gly Asn
770 775 780

Phe Thr Glu Ser Thr Asp Gly Gly Lys Ala Ile Leu Lys Asn His Leu
785 790 795 800

Asp Gln Asn Pro Gln Leu Gln Trp Asn Thr Thr Glu Pro Ser Arg Thr
805 810 815

Cys Lys Asp Pro Ile Glu Asp Ile Asn Ser Pro Glu His Ile Gln Arg
820 825 830

Arg Leu Ser Leu Gln Leu Pro Ile Leu His His Ala Tyr Leu Pro Ser
835 840 845

Ile Gly Gly Val Asp Ala Ser Cys Val Ser Pro Cys Val Ser Pro Thr
850 855 860

Ala Ser Pro Arg His Arg His Val Pro Pro Ser Phe Arg Val Met Val
865 870 875 880

Ser Gly Leu